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Combined effects of hydrology and manure on the vegetation of Mediterranean temporary ponds in the Moroccan Middle Atlas

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ABSTRACT

Mediterranean temporary ponds are unique ecosystems characterized by alternating flooding and drying phases, which support highly specialized plant communities. However, these habitats are increasingly threatened by environmental pressures that may alter their ecological functioning. The aim of this study was to evaluate the effects of hydrological conditions and manure on the vegetation of Mediterranean temporary ponds in the Moroccan Middle Atlas. Sediments collected from four temporary ponds were used in a microcosm experiment. Different manure treatments and hydrological regimes (flooded, moist, and dry) were applied to assess their effects on plant density, species richness, and floristic composition. The results showed that manure significantly affected total plant density as well as the density of aquatic, annual aquatic, and perennial aquatic plants, while no significant effect was observed on species richness. In contrast, hydrology had a strong influence on density, species richness, and plant community composition. Floristic composition analyses also revealed significant effects of manure, hydrology, and their interaction. These findings highlight the key role of hydrological conditions in maintaining biodiversity and ecological functioning in Mediterranean temporary ponds, whereas manure mainly influences plant abundance. This study improves our understanding of the ecological processes governing these habitats and provides useful information for their conservation and sustainable management.

Keywords: Mediterranean temporary ponds; hydrology; manure; species richness; plant density; Middle Atlas.

INTRODUCTION

Temporary ponds constitute ecologically distinctive wetlands, characterized by a seasonal alternation between flooding phases and drying periods over the annual cycle. This cyclical dynamic, closely linked to rainfall regimes and thermal fluctuations, profoundly conditions the functioning of these ecosystems as well as the evolution of their biological communities (Grillas et al., 2004). Generally located in closed depressions, these small water bodies are fed mainly by autumn and winter precipitation before drying out gradually over the summer. This succession between aquatic and terrestrial states gives temporary ponds a distinctive ecological identity, giving rise to dynamic and heterogeneous habitats.

The Mediterranean basin, and Morocco in particular, still harbors numerous temporary ponds (Thiéry, 1987; Rhazi et al., 2001; Bélair,

2005; Ferchichi-Ben Jamaa et al., 2010; Madihi et al., 2026), which provide important ecosystem services to local populations (Bouahim et al., 2014; Boudjaj et al., 2026a). These environments host considerable plant (Grillas et al., 2004; Rhazi et al., 2005; Muller et al., 2022) and animal (Thiéry, 1987; Van den Broeck et al., 2015) biodiversity, including numerous endemic, rare, or threatened taxa, some of which are listed on the IUCN Red List for North Africa (García et al., 2010). However, their small surface area and shallow depth make them particularly vulnerable to degradation, or even disappearance (Biggs et al., 2005; Van den Broeck et al., 2016; Ben Bammou et al., 2021; Boudjaj et al., 2026b). These ecosystems are thus declining markedly under the effect of increasing anthropogenic pressures (Rhazi et al., 2012), even though they are among the richest habitats in biodiversity at the global scale (Dudgeon et al., 2002).

These habitats are today among the most threatened freshwater ecosystems, owing to the combined effect of intensifying human activities (Deil, 2005; Javornik & Collinge, 2016) and the consequences of climate change (Rosset et al., 2010; Ewald et al., 2013; Madihi et al., 2026). Several global and regional analyses have moreover identified the Mediterranean region as a zone particularly sensitive to climate change and vulnerable to its impacts (Giorgi & Lionello, 2008; Cramer et al., 2018; Moukrim, et al., 2026). According to Giorgi (2006), the Mediterranean even constitutes the second most pronounced climate change hotspot at the global scale. Most general circulation models agree on a temperature increase across all seasons and throughout the Mediterranean rim by the end of the 21st century (Dubrovský et al., 2014), while marked warming, reduced precipitation, and increased interannual rainfall variability are expected to be particularly pronounced in summer (Zachariadis, 2016).

These changes are likely to have profound effects on both terrestrial and aquatic habitats (Cramer et al., 2018), and the productivity of temporary ponds appears particularly exposed to this risk (Johnson et al., 2005). Emergent plant communities, indeed, respond most directly to the hydrological alterations induced by climate change (Short et al., 2016). The response of plant growth to reduced precipitation depends on pre-existing factors such as salinity and soil saturation level, with such a reduction sometimes improving and sometimes decreasing productivity (Short et al., 2016). Higher temperatures would furthermore lead to lower water levels and a greater abundance of drought-tolerant species in freshwater marshes, with a risk of seasonally flooded wetlands shifting toward regions that are currently wetter (Johnson et al., 2005; Moukrim et al., 2025). The repercussions of climate change on these environments will thus depend closely on how water deficits evolve, which already vary considerably from one region of the Mediterranean basin to another.

Beyond climate, many temporary ponds also serve as grazing areas (Bouahim et al., 2015; Croel & Kneitel, 2011a; Schmutzer et al., 2008), which has led researchers to closely examine the impact of grazing on the composition of plant and animal communities, as well as on the overall functioning of these ecosystems (Marty, 2015; Pyke & Marty, 2005; Rhazi et al., 2006; Bagella et al., 2010; Bouahim et al., 2010; Boughton et al., 2016). Depending on its intensity, which varies along a gradient, grazing exerts contrasting effects on pond biodiversity, sometimes positive and sometimes negative (Schmutzer et al., 2008; Bouahim et al., 2010; Epele & Miserendino, 2015; Davidson et al., 2017). Moderate grazing, corresponding to low livestock densities at a given time, is thus frequently adopted as a wetland management measure aimed at preserving or restoring floristic diversity (Gordon et al., 1990; Rhazi et al., 2005; Dumont et al., 2007).

Livestock, however, tend to concentrate around littoral zones to feed there (Foote & Hornung, 2005; James et al., 2007), sometimes going as far as entering the ponds directly to graze, environments that nonetheless harbor a rare and fragile flora, including threatened species (Barbour et al., 2007; Rhazi et al., 2012; Bouahim et al., 2014). Yet the resulting animal excrement represents a major threat to watersheds, shallow water bodies, and ponds, through organic and nutrient enrichment effects associated with algal proliferation (Carpenter et al., 1998; Belsky et al., 1999; Smith et al., 1999; Anderson et al., 2002).

In Morocco, as in other Maghreb countries, temporary ponds are commonly exploited by livestock. This pastoral use is accompanied by a variable input of manure, whose specific impact on the vegetation characteristic of these habitats (often dominated by short-cycle annual species) has so far received little direct experimental attention.

The influence of hydrological regime on temporary pond vegetation, and the effects of organic enrichment linked to grazing, are relatively well established in the literature when considered separately. However, how these two factors interact , and in particular the specific role of manure deposited by livestock under contrasting hydrological conditions, has received comparatively little experimental evaluation, in North African temporary ponds in particular. While the factorial, manipulative approach used here provides original experimental evidence on this specific interaction, the individual effects of organic enrichment and of hydrological regime on wetland vegetation are already well established in the literature; the conceptual novelty of the study is therefore considered modest, its main contribution lying in the experimental quantification of their combined effects for the temporary ponds of the Moyen Atlas. In a context where climate change is already altering the duration and intensity of the flooding phases of these environments, an experimental assessment of this interaction is needed to clarify how organic input, combined with hydrological constraints, influences the richness, abundance, and composition of plant communities in these fragile and threatened ecosystems. It is within this perspective, and specifically for the temporary ponds of the Moyen Atlas, that the present study is framed, with the following objectives:

1. To investigate the effect of hydrology and manure on plant species richness and abundance in temporary ponds;
2. To analyze the impact of hydrology and manure on the composition of plant communities in temporary ponds.

Study area

The study targeted several temporary ponds distributed across different areas of the Middle Atlas, notably Ifrane, Azrou, and Timahdite (Fig. 1). Oriented northeast–southwest, the Middle Atlas forms the central element of the Moroccan mountain range. It is bounded to the north by the Taza corridor, which separates it from the Rif; to the south by the High Atlas and the upper Moulouya; to the east by the middle Moulouya valley; and to the west by the Central Plateau (Touabay et al., 2002).

This massif is characterized by diverse forest cover and a set of wetlands, both permanent and, above all, temporary, described as floristic jewels by Braun-Blanquet.

The central Middle Atlas is characterized by a cold, humid climate, classified as a cold montane Mediterranean climate, owing to its altitude, geographic setting, and oceanic influence (Martin, 1981). Abundant precipitation is generated mainly by air masses arriving from the west (Lamb & Peppler, 1991; Cheddadi et al., 1998). Two types of wind dominate depending on the season: the Gharbi, a humid wind prevailing in winter, and the Chergui, a dry, hot wind, often laden with Saharan dust in summer (Baali, 1998).

In winter, Atlantic disturbances meet the Middle Atlas relief, causing cloud build-up and heavy precipitation; as the air descends, it dries out (Bentayeb & Leclerc, 1977). Precipitation is concentrated mainly between October and April, with about 70 days of rain per year, but it shows strong interannual variability and can include intense events with significant ecological impacts (Lamb & Peppler, 1991; Baali, 1998; Neijari et al., 2001).

The flooding duration and maximum depth of the studied ponds are subject to substantial interannual variation depending on each year's climatic conditions and, consequently, on the amount of precipitation recorded.

Experimental design

The experiment was conducted in microcosms using soil collected on 18 November 2019 from four temporary ponds in the Middle Atlas (Supplementary Table 11)., in order to constitute a diaspore bank representative of regional biodiversity. At the time of sampling, the ponds were still dry (Fig. 1) and located in rocky areas inaccessible to grazing, which rules out any influence of manure quantities that could bias the study results. Sixty independent soil samples were collected from each pond using a metal auger (4 cm depth × 20 cm diameter). Sampling was restricted to the top 4 cm of soil, a layer recognized for its richness and high density of diaspores (seeds, spores, and other resistance propagules) (Rhazi et al., 2001). The seeds of temporary pond species are characterized by remarkable longevity and can persist in the soil for several years, or even several decades (Zedler, 2000; Grillas et al., 2004; Rhazi et al., 2005).

After collection on 18 November 2019, the samples were stored in airtight plastic bags, in the dark and at room temperature, until the start of the germination experiment in January 2022.

The experimental unit in this study was an individual germination tray with a surface area of 0.16 m². For each pond, the 60 independent soil samples were divided into four manure treatments (Figure 2). The first series served as the control (0g), whereas the second, third, and fourth series were subjected to applications of sheep manure at rates of 35g (fresh sheep manure representing the average amount of waste per equivalent unit area in natural ponds (Van den Broeck et al., 2019), 17,5g (50% of the average amount to simulate moderate grazing), and 52.5g (the average amount plus 25% to simulate extensive grazing).

Fifteen trays were assigned to each manure treatment, and each manure treatment was subjected to three hydrological conditions: Dry (D), Wet (W), and Flooded (F), with five trays assigned to each hydrological treatment. This resulted in a fully factorial experimental design of 4 × 4 × 3 × 5 = 240 trays (4 ponds × 4 manure treatments × 3 hydrological treatments × 5 replicates). In each tray, the soil samples were spread to a depth of 3 cm (Figure 2).

Sheep manure was selected because sheep are the most widespread livestock in Moroccan temporary ponds (Bouahim et al., 2014). Fresh sheep manure was obtained from a local livestock farmer in January 2022. Its physicochemical characteristics were not determined in this study. Because the nutrient content of the manure was not quantified, treatments are expressed as fresh manure mass per unit area rather than as nutrient loads. The effect of hydrology was tested for each of the four manure treatments, with five replicates per treatment, according to three hydrological conditions (Rhazi et al., 2009):

- Flooded – trays were kept fully flooded, with a 3 cm water layer maintained above the soil surface. Water level was readjusted every two weeks by adding distilled water. Given the surface area of each tray (0.16 m²), this corresponds to an applied water volume of approximately 4.8l per tray.
- Wet – trays were watered to keep the soil at saturation, using 800 ml of water per tray.
- Dry – trays were lightly watered by hand twice a week, using 100 ml of water per tray. This treatment therefore represents reduced watering rather than a complete absence of water.

All soil samples were subjected to germination conditions from January 2022 to June 30, 2022, at a temperature of 18 °C and under an 8/16 h photoperiod. The full experimental period, from the start of germination monitoring to the end of the experiment, therefore corresponded to approximately 24 weeks (January–June 2022).

Every 15 days, plants germinating from the seeds were identified and subsequently removed. Species names were assigned using the Practical Flora of Morocco (Fennane and Ibn Tattou 2005). Taxonomic identifications, particularly for rare or protected species, were verified by following the *Catalogue des plantes endémiques, rares ou menacées du Maroc* (Fennane and Ibn Tattou 1998).

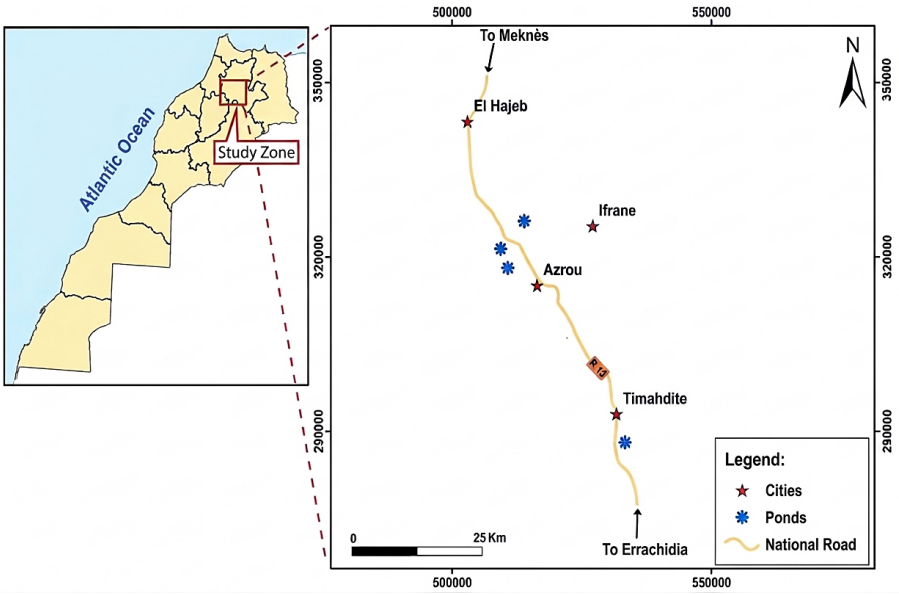

Figure 1. Location map of sampled temporary ponds

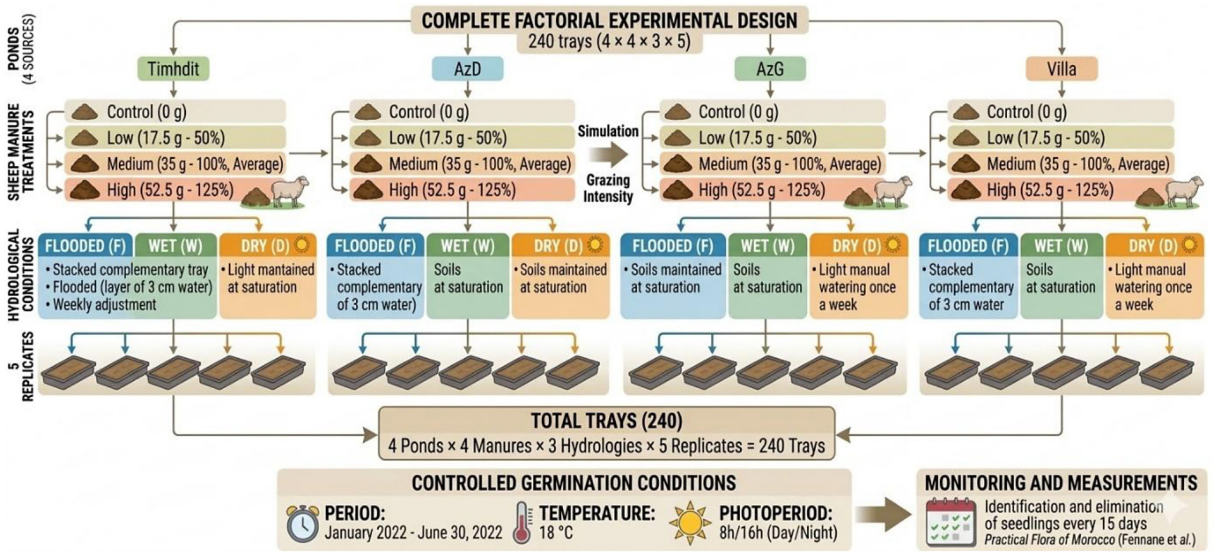

Figure 2. Experimental design illustrating soil sampling and the hydrological and manure treatments applied to temporary ponds

Data analysis

Data normality was assessed using the Shapiro–Wilk test, while homogeneity of variances was evaluated using Levene's test. To assess the effects of hydrology and manure addition on the different vegetation parameters, a two-way analysis of variance (two-way ANOVA) was performed. This approach allowed us not only to test the main effect of each factor independently but also to detect a potential interaction between the two factors, i.e., to determine whether the effect of manure on vegetation varied according to the hydrological regime considered. Throughout this manuscript, we use the term "germinated propagule density", rather than "seed density," because the variable actually measured corresponds to the number of seedlings that emerged and were identified from the soil propagule bank, not a direct count of seeds present in the soil.

To formally address this hierarchical sampling structure, the two-way ANOVA described above was complemented with linear mixed-effects models fitted in R (R Core Team) using the lme4 package (Bates et al., 2015). For each response variable (germinated propagule density and species richness, for the total flora and for each species group), hydrology, manure addition, and their interaction were specified as fixed effects, and pond identity (n = 4 ponds) was included as a random intercept to account for the non-independence of the 60 germination trays nested within each pond. Statistical significance of fixed effects was assessed using Type III F-tests with Satterthwaite's approximation for denominator degrees of freedom, implemented in the lmerTest package (Kuznetsova et al., 2017).

To examine the relationship between germinated propagule density and species richness for each species group considered, a non-parametric correlation test based on Spearman's correlation coefficient (Spearman's rho) was used.

Regarding the floristic composition of plant communities, a permutational multivariate analysis of variance (PERMANOVA), based on 9999 permutations, was subsequently performed to test the effects of hydrological and manure treatments, as well as their interaction, on the overall structure of plant communities.

The original PERMANOVA (9999 permutations) did not constrain permutations by the structure of the four source ponds. The analysis was therefore rerun with the adonis2 function of the vegan package (R), using permutations restricted within ponds (strata = Pond) to account for the nested sampling design and avoid pseudo-replication in the significance testing, and keeping the same Bray–Curtis dissimilarity and the same model (hydrology × manure). The results obtained under this restricted permutation scheme were consistent with those of the original unconstrained analysis: hydrology ($F = 35.796$; $P = 0.0001$), manure addition ($F = 4.850$; $P = 0.0001$), and their interaction ($F = 2.584$; $P = 0.0001$) all remained statistically significant predictors of plant community composition.

Finally, to identify the species responsible for the observed dissimilarities among communities subjected to contrasting environmental conditions, a SIMPER (Similarity Percentage) analysis was performed. Prior to this analysis, species abundance data were transformed using the logarithmic function $\log_{10}(x + 1)$, a standard transformation in community ecology that reduces the disproportionate influence of highly abundant species while retaining the contribution of rare species.

The SIMPER procedure therefore allowed us to quantify, for each species, its contribution to the overall dissimilarity between the groups being compared, thereby identifying the most discriminating taxa among the different environmental conditions tested.

The statistical analyses, including two-way analyses of variance (two-way ANOVA), Spearman's correlation analysis, and the SIMPER analysis, were performed using PAST software, version 2.0 (Hammer et al., 2001). The PERMANOVA analysis, as described above, was performed in R (R Core Team) using the adonis2 function of the vegan package. Both PERMANOVA and SIMPER analyses were conducted based on the Bray–Curtis dissimilarity index.

For the linear mixed-effects models, model residual degrees of freedom were 226 for all response variables based on the full dataset (n = 240), and 213 (species richness) or 222 (germinated propagule density) for aquatic perennial species, reflecting the trays with no aquatic perennial individuals that were excluded from that analysis. Partial eta-squared (η^2p) effect sizes were computed for each fixed effect from the Type III F-tests, together with one-sided 95% confidence intervals (upper bound fixed at 1, following Steiger, 2004; package effectsize, R), and are reported in (Supplementary Table 5).

Regarding the treatment of the pond factor: pond identity (n = 4) was included in each mixed model as a random intercept rather than as a fixed blocking factor (see Methods), because the primary aim was to estimate the fixed effects of hydrology and manure addition generically across ponds, rather than pond-specific effects; the proportion of variance attributable to pond identity for each response variable is quantified by the intraclass correlation coefficients (ICC) already reported in (Supplementary Table 5).

In addition to the PERMANOVA reported above (Table 2), the homogeneity of multivariate dispersions among groups was tested using the betadisper function (package vegan), based on the same Bray–Curtis dissimilarity matrix, with significance assessed via 9999 permutations. Dispersions differed significantly among the three hydrological regimes ($F = 53.51$; $df = 2.237$; $P = 0.0001$), indicating that part of the significant PERMANOVA effect of hydrology on community composition may reflect differences in within-group variability (dispersion) rather than differences in community centroids alone (see Discussion). In contrast, dispersions did not differ significantly among the four manure levels ($F = 2.12$; $df = 3.236$; $P = 0.100$), supporting a straightforward centroid-shift interpretation of the manure effect on floristic composition. Dispersions also differed significantly among the twelve hydrology × manure treatment combinations ($F = 9.07$; $df = 11, 228$; $P = 0.0001$), a pattern consistent with the hydrology-driven dispersion difference identified above.

RESULTS

The two-way analyses of variance (two-way ANOVA), testing the effects of hydrology, manure addition, and their interaction, revealed contrasting effects depending on the variable considered (density vs. species richness) and the species group analyzed (all species, aquatic species, annual aquatic species, and perennial aquatic species).

Regarding species density, both factors had highly significant effects across all species groups considered. The effect of hydrology was consistently stronger than that of manure addition, reaching its maximum for perennial aquatic species ($F = 51.402$, $P < 0.001$) (Table 1). The Hydrology × Manure interaction was also significant for all species groups, indicating that the effect of manure addition on species density varied according to the hydrological regime.

In contrast, regarding species richness, hydrology had a highly significant effect across all species groups, whereas manure addition had no significant effect on species richness, regardless of the species group considered (Table 1b). The Hydrology × Manure interaction was significant only for annual aquatic species ($F = 2.531$, $p = 0.022$).

When pond identity was included as a random effect to account for the hierarchical, nested sampling structure (60 germination trays nested within each of 4 source ponds), a substantial proportion of the variance in both germinated propagule density and species richness was attributable

Table 1. Results of two-way analyses of variance testing the effects of hydrology, manure addition, and their interaction on germinated propagule density (a) and species richness (b) across different species groups. The significance level was set at $P < 0.05$

Variables	a) Species density			b) Species richness		
	DF	F value	P value	DF	F value	P value
Totality of species						
Hydrology	2	4.930	0.002	2	0.383	0.001
Manure	3	32.050	0.000	3	7.026	0.765
Hydrology*Manure	6	2.954	0.009	6	0.955	0.456
Aquatic species						
Hydrology	2	5.361	0.001	2	0.217	0.000
Manure	3	37.413	0.000	3	14.590	0.884
Hydrology*Manure	6	3.378	0.003	6	1.951	0.074
Aquatic annual species						
Hydrology	2	8.166	0.000	2	0.057	0.000
Manure	3	21.334	0.000	3	13.688	0.982
Hydrology*Manure	6	4.758	0.000	6	2.531	0.022
Aquatic perennial species						
Hydrology	2	2.904	0.036	2	0.043	0.000
Manure	3	51.402	0.000	3	2.473	0.897
Hydrology*Manure	6	2.859	0.011	6	0.038	0.987

to pond identity (ICC ranging from 0.18 to 0.61 across species groups), confirming that samples from the same pond were not fully independent (Supplementary Table 6). After accounting for this structure, the effects of hydrology and of the hydrology × manure interaction on germinated propagule density and species richness remained statistically significant for most species groups, consistent with the original two-way ANOVA. The main effect of manure addition remained significant, although attenuated, for germinated propagule density in all species groups, and remained non-significant for species richness in all species groups, as in the two-way ANOVA (Supplementary Table 5). The effect of manure on germinated propagule density is therefore robust to the correction for pond identity.

Effect of organic matter on germinated propagule density

The total germinated propagule density (F = 4.93; df = 3; $P = 0.002$), as well as the densities of aquatic species (F = 5.36; df = 3; $P = 0.001$), annual aquatic species (F = 8.166; df = 3; $P < 0.001$), and perennial aquatic species (F = 2.904; df = 3; P = 0.036), varied significantly according to the amount of organic matter added.

Total germinated propagule density, as well as the density of aquatic species and annual aquatic species, was significantly higher under the 35g and 17.5g treatments, which did not differ significantly from each other (Figure 3a, 3c). In contrast, the germinated propagule density of perennial aquatic species increased significantly with increasing amounts of organic matter (Figure 3d).

Effect of Hydrology on germinated propagule density

The total germinated propagule density (F = 32.05; df = 2; $P < 0.00001$), as well as the densities of aquatic species (F = 37.41; df = 2; $P < 0.00001$), annual aquatic species (F = 21.33; df = 2; $P < 0.00001$), and perennial aquatic species (F = 51.402; df = 2; $P < 0.00001$), differed significantly among the three hydrological treatments.

Total germinated propagule density and aquatic species density were significantly higher under the Wet (W) and Flooded (F) treatments, which did not differ significantly from each other. The density of annual aquatic species was significantly higher under the Wet treatment but lower under the Dry and Flooded treatments, which did not differ significantly from each other ($P > 0.05$) (Figure 4a-c). In contrast, the density of perennial

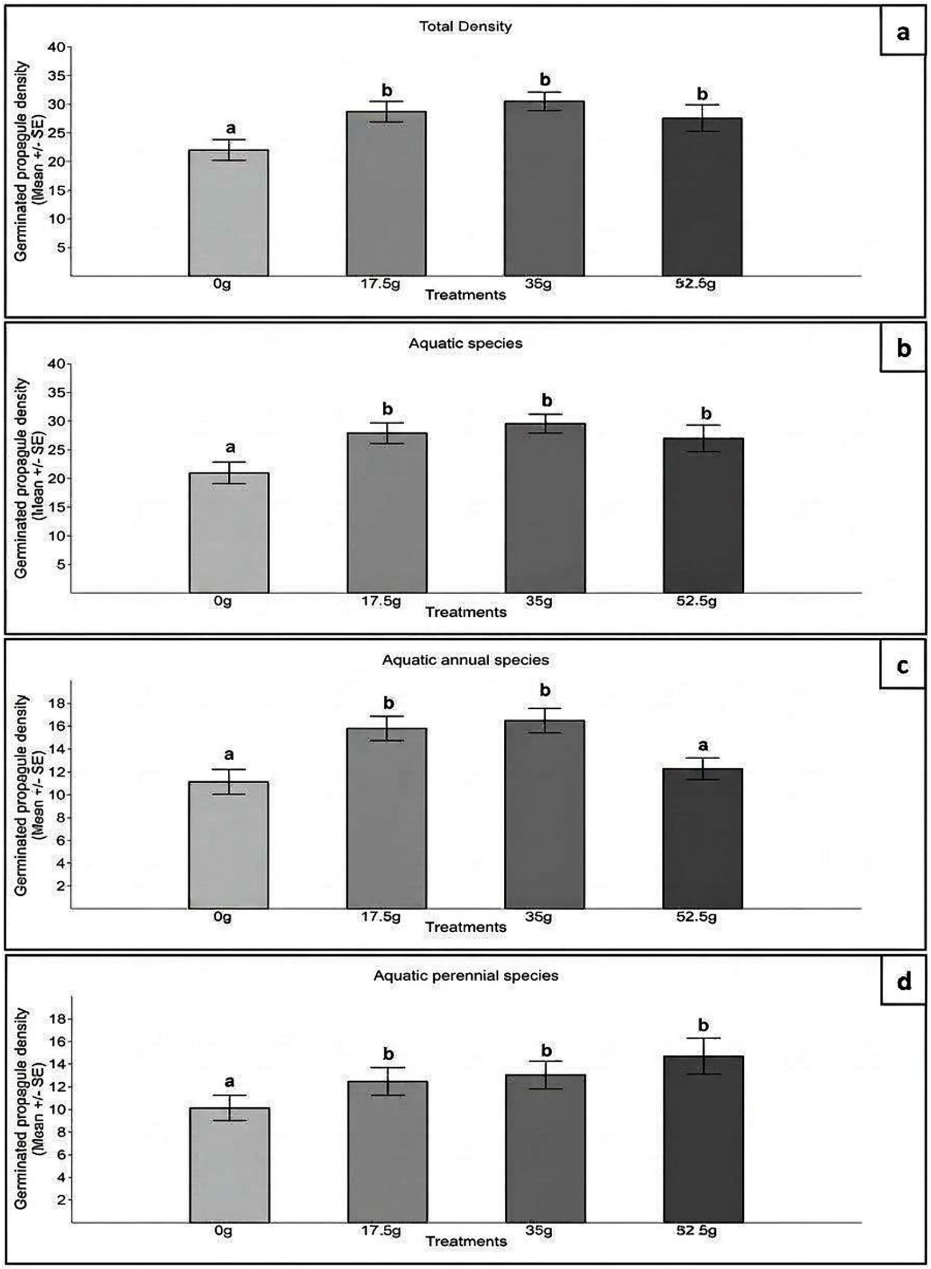

Figure 3. Results of the one-way analysis of variance (ANOVA-1) comparing germinated propagule density among manure treatments (0, 17.5, 35 and 52.5g) for different species groups
Different letters above columns indicate significantly different means (Tukey–Kramer test, $P < 0.05$)

aquatic species increased significantly with increasing hydrological conditions (Figure 4d).

Effect of hydrology on species richness

Total species richness (F = 7.026; df = 2; P = 0.001), as well as the richness of aquatic species (F = 14.59; df = 2; $P < 0.00001$), annual aquatic species (F = 13.69; df = 2; $P < 0,00001$), and perennial aquatic species (F = 10.33; df = 2; $P <$ 0.00001), differed significantly among the three hydrological treatments.

Total species richness and aquatic species richness were significantly higher under the Wet (W) and Flooded (F) treatments, which did not differ significantly from each other. The richness of annual aquatic species was significantly higher under the Wet treatment but lower under the Dry and Flooded treatments, which did not differ significantly from each other ($P > 0,05$) (Figure 5a-c). In contrast, the richness of perennial aquatic species was significantly higher under the Flooded (F) treatment, whereas it was lower under the dry (D) and Wet (W) treatments,

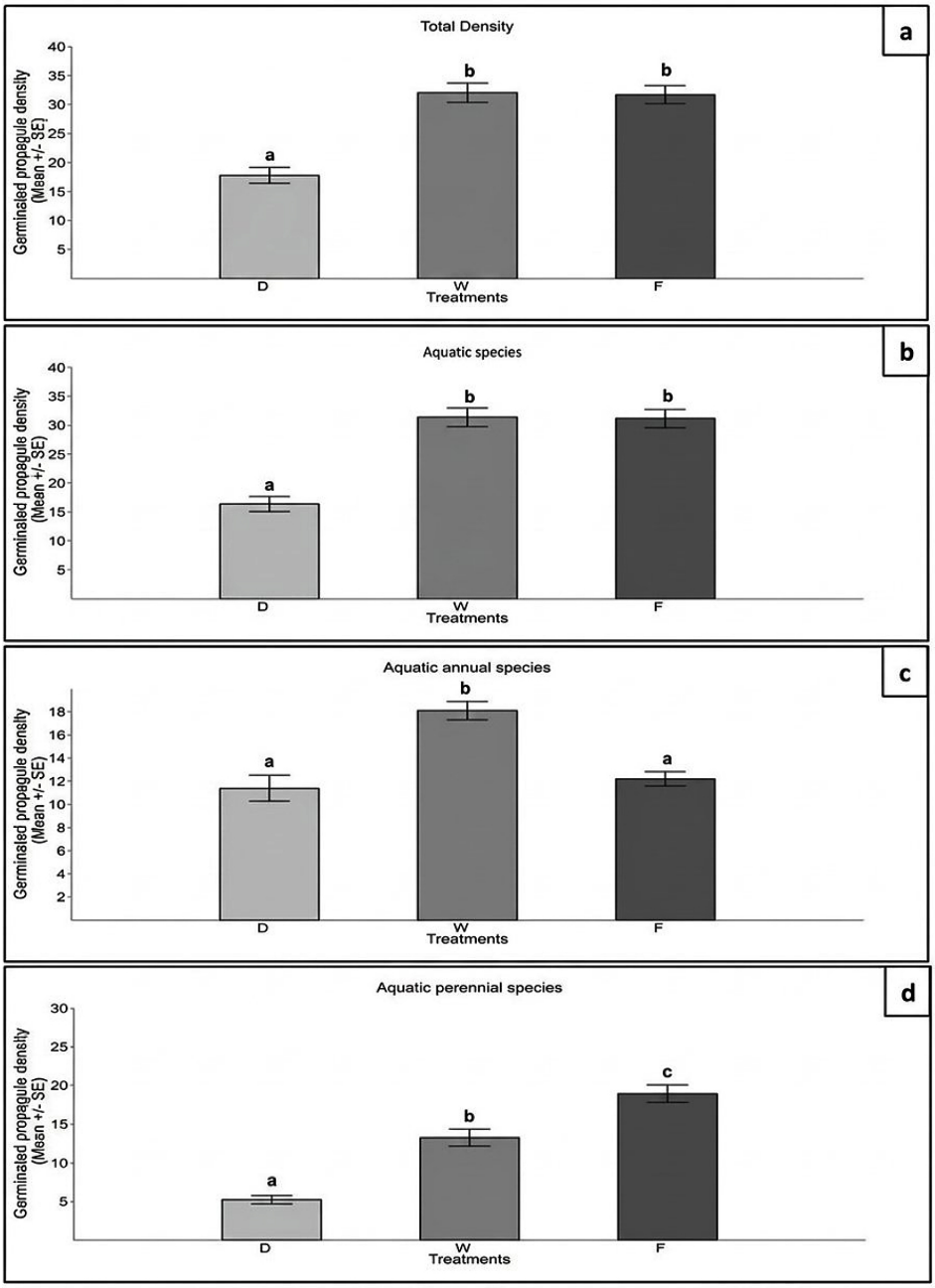

Figure 4. Results of the one-way analysis of variance (ANOVA-1) comparing germinated propagule density among hydrological treatments (D: dry, W: wet, and F: flooded) for different species groups
Different letters above columns indicate significantly different means (Tukey–Kramer test, $P < 0.05$).

which likewise did not differ significantly from each other (Figure 5d).

Effect of organic matter on species richness

Total species richness ($F = 0.383$; $df = 3$; $P = 0.765$), as well as the richness of aquatic species ($F = 0.217$; $df = 3$; $P = 0.884$), annual aquatic species ($F = 0.057$; $df = 3$; $P = 0.982$), and perennial aquatic species ($F = 0.198$; $df = 3$; $P = 0.897$), did not differ significantly among the four manure treatments (Figure 6).

Correlation between germinated propagule density and species richness

Germinated propagule density and species richness were significantly and positively correlated across all species groups considered (Figure 7). This positive relationship should, however, be interpreted with caution, as it partly reflects a

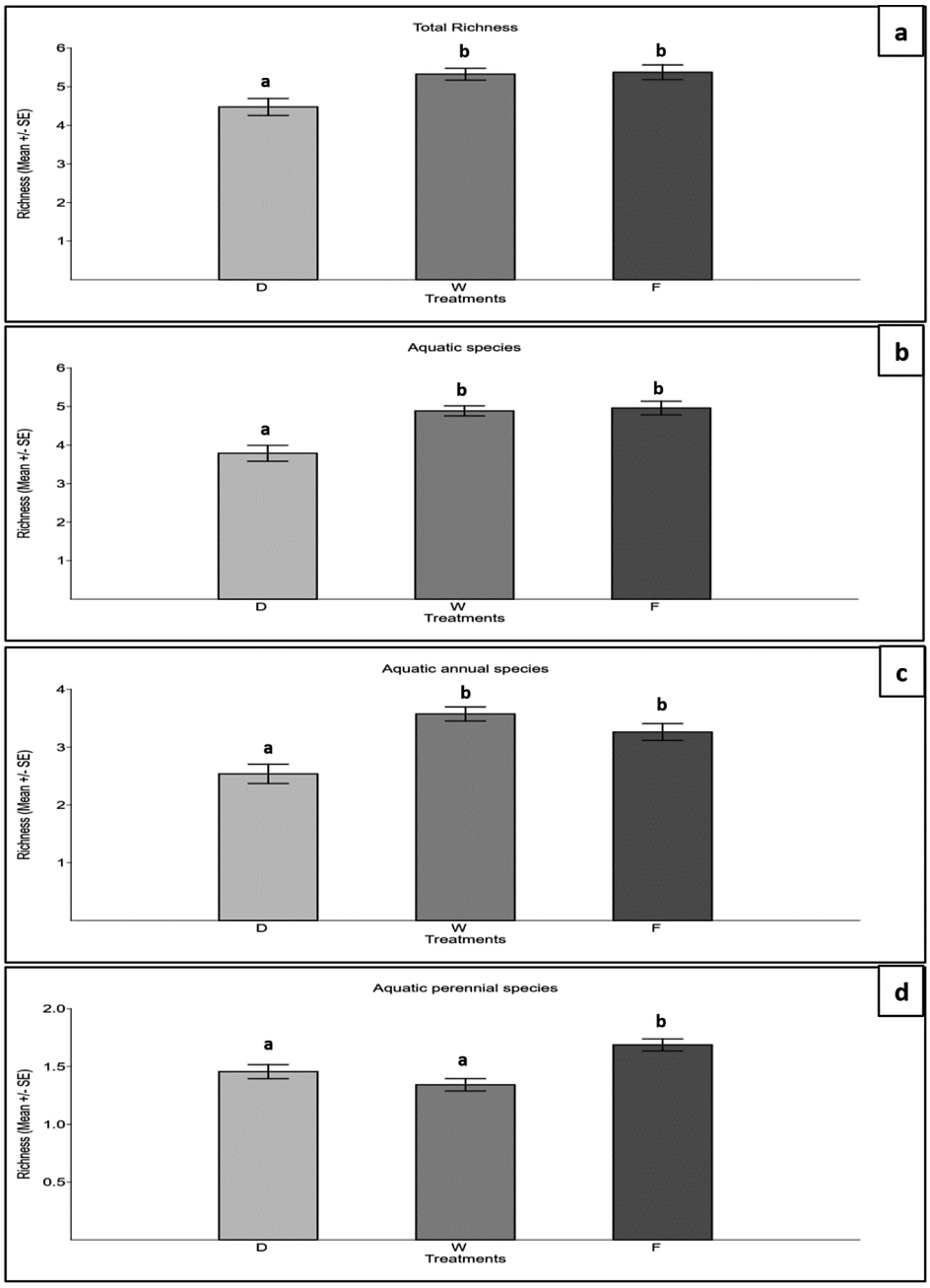

Figure 5. Results of the one-way analysis of variance (ANOVA-1) comparing species richness among hydrological treatments (D: dry, W: wet, and F: flooded) for different species groups
Different letters above columns indicate significantly different means (Tukey–Kramer test, $P < 0,05$).

mathematical dependency between the two measures: a higher number of germinated propagules mechanically increases the probability of detecting a greater number of species, independently of any additional ecological mechanism.

Plant community composition

With 55,75% of the inertia explained by Axis 1 and 19% by Axis 2 (74,75% cumulative), this factorial plane offers a reasonably informative, though purely descriptive, representation of compositional variation; in the absence of a direct statistical association with measured environmental variables, this ordination should be read as illustrative rather than confirmatory (Figure 8). Axis 1 separates samples dominated by the control treatment (circles) and low manure additions (squares) from those with the highest manure addition (crosses), together with the isolated species *Isoetes velata*, associated here with a control sample under flooded conditions. This pattern is consistent

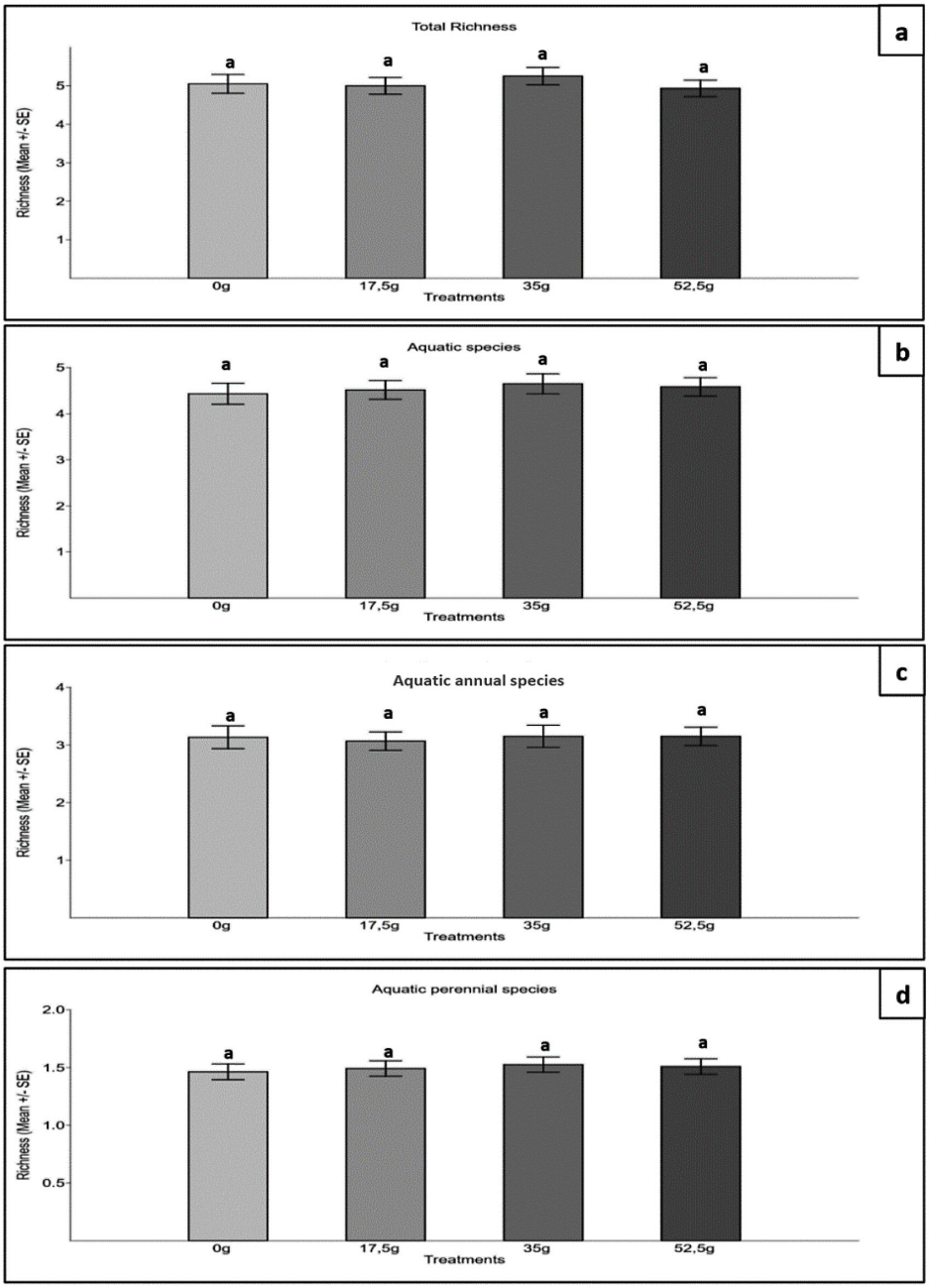

Figure 6. Results of the one-way analysis of variance (ANOVA-1) comparing species richness among manure treatments (0, 17.5, 35 and 52.5g) for different species groups
Different letters above columns indicate significantly different means (Tukey–Kramer test, $P < 0,05$).

with the hypothesis that Axis 1 could reflect a gradient of organic enrichment, with plant communities progressively differentiating as manure addition increases; this interpretation would, however, need to be corroborated by an explicit correlation with measured soil or water nutrient variables.

Axis 2 appears more closely related to the hydrological regime. The ellipse corresponding to the W (wet) treatment extends toward the highest values on the graph, coinciding with crosses and squares representing higher manure additions under wet conditions; this co-occurrence may tentatively suggest an association between *Damasonium bourgaei, Eleocharis palustris*, and *Ranunculus batrachioides* and wet, organically enriched conditions, without implying a direct indicator or causal relationship. Conversely, the D (dry) treatment remains concentrated near the origin, within a narrower ellipse, indicating a more homogeneous plant community that is comparatively less responsive to the manure gradient. The F (flooded) treatment is more widely distributed along Axis 1

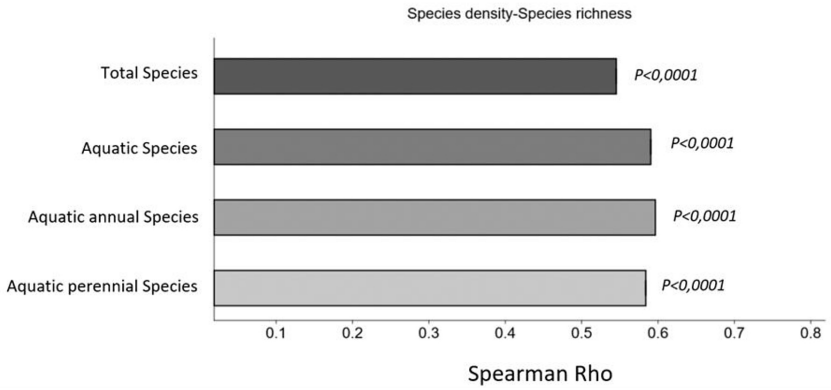

Figure 7. Spearman's nonparametric correlation between germinated propagule density and species richness across the different species groups

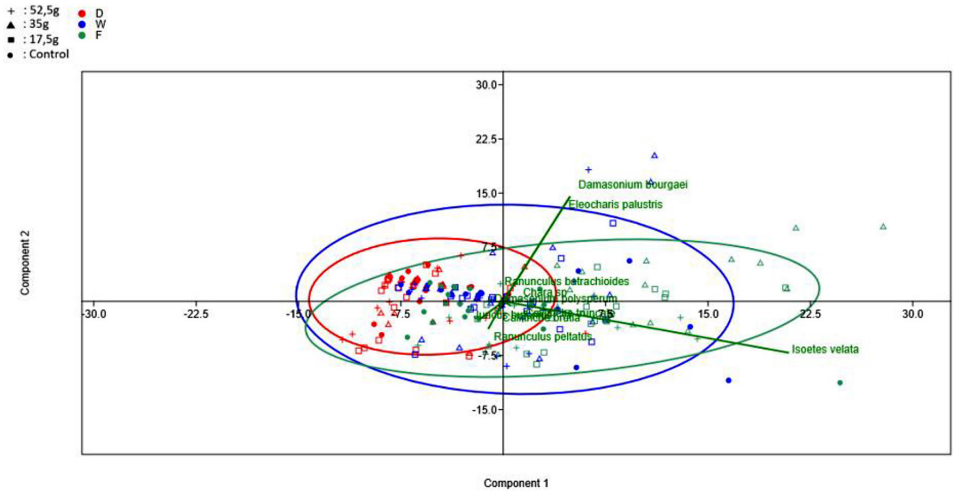

Figure 8. Results of the correspondence analysis (CA) showing species and the centroids of the hydrological treatments D, W, and F, and the manure treatments 0g, 17.5g, 35g, and 52.5g

and extends to *Isoetes velata* at the rightmost end, a species generally characteristic of oligotrophic, relatively undisturbed temporary ponds, consistent with, though not proof of, its proximity to a control sample without manure addition.

Considering both factors together, a relatively clear interaction between hydrology and manure addition can therefore be observed. The most pronounced changes in community composition occur under wet or flooded conditions as manure addition increases, whereas under dry conditions, vegetation composition appears to remain relatively stable regardless of the level of organic amendment. Nevertheless, the substantial overlap of the three ellipses around the center of the graph indicates that a considerable proportion of the samples, particularly those corresponding to the control and low manure treatments, do not clearly

Table 2. Results of the PERMANOVA comparing plant community composition according to hydrological treatment (Dry, Wet, and Flooded), manure addition (0g, 17.5g, 35 g, and 52.5g), and their interaction

Source	Sum of squares	Df	Mean square	F	p
Manure	1.87592	3	0.62531	12.268	0.0001
Hydrology	6.43459	2	3.2173	63.121	0.0001
Interaction	1.64215	6	0.27369	5.3696	0.0001
Residual	11.6212	228	0.05097		
Total	21.574	239			

differ among the three hydrological regimes in this factorial plane.

Hydrology had a highly significant effect on plant community composition (F = 35.796; P = 0.0001). Thus, species assemblages differed significantly among the different hydrological regimes. Manure addition also had a significant effect (F = 4.850; P = 0.0001), indicating that changes in manure quantity altered the floristic composition of temporary ponds. Consequently, some species appear to be favored or disadvantaged depending on the amount of manure added to the soil.

The Hydrology × Manure interaction was significant (F = 2.584; P = 0.0001). This indicates that the effect of manure addition was not consistent across hydrological conditions. In other words, the influence of manure addition on plant community composition depended on the hydrological regime considered.

Effect of the hydrological regime

The Similarity Percentage (SIMPER) analysis attributed these differences to an overall mean dissimilarity of 42.9% among the three hydrological regimes (Dry, Wet, and Flooded), corresponding to a residual similarity of approximately 57%. *Isoetes velata* was the main discriminating taxon, accounting for 29.6% of the total dissimilarity on its own. Its mean abundance increased markedly along the flooding gradient, from 3.75 under Dry conditions to 1.5 under Wet conditions and 15.4 under Flooded conditions (Table 3). This community structuring reflects niche partitioning along the moisture gradient. *Damasonium bourgaei* and *Eleocharis palustris*, which had identical abundances in this analysis, increased from 3.25 under Dry conditions to 3.76 under Wet conditions and 5.60 under Flooded conditions, accompanying *Isoetes velata* in areas subjected to longer periods of inundation (Table 3).

In contrast, *Callitriche truncata* (5.88 under Wet conditions, compared with 2.05 and 2.23 under the two extreme conditions) and, to a lesser extent, *Ranunculus peltatus* (with an optimum under Wet conditions : 6.58) exhibited their highest abundances under intermediate conditions. Finally, *Juncus bufonius*, a pioneer species of drained substrates, decreased with increasing inundation, with its abundance declining from 3.16 under Dry conditions to 1.91 under Flooded conditions. The five leading taxa together accounted for nearly 81% of the total dissimilarity, whereas *Ranunculus batrachioides* remained ubiquitous and non-discriminating, accounting for only 0.17% (Table 4).

Effect of manure quantity

For a comparable overall mean dissimilarity (40.3%), *Isoetes velata* remained the main contributor (27.11%), with its abundance increasing monotonically with manure addition, from 8.57 in the control treatment to 11,4 under the highest manure treatment (Table 4). The most pronounced pattern, however, was observed for *Ranunculus peltatus* (14.72% of the dissimilarity), which exhibited a unimodal response: its abundance peaked at the low and intermediate manure additions (7.02 and 7.00 respectively) before declining sharply at the highest addition (4.07), falling even below the control value (4.63). A similar pattern characterized *Callitriche truncata*, *Callitriche brutia*, and *Juncus*

Table 3. Contribution of species to floristic dissimilarity among the Dry, Wet, and Flooded treatments based on SIMPER analysis (Bray–Curtis index)

Taxon	Contrib. %	Cumulative %	Mean sec	Mean humide	Mean
Isoetes velata	29.57	29.57	3.75	11.5	15.4
Ranunculus peltatus	13.50	43.06	5.95	6.58	4.51
Damasonium bourgaei	13.45	56.51	3.25	3.76	5.6
Eleocharis palustris	13.45	69.96	3.25	3.76	5.6
Callitriche truncata	10.82	80.78	2.05	5.88	2.23
Juncus bufonius	8.30	89.08	3.16	3.03	1.91
Callitriche brutia	5.52	94.6	1.78	2.36	2.13
Damasonium polysperum	2.79	97.39	1.1	1.85	1.38
Chara sp.	2.44	99.83	1.09	1.63	1.45
Ranunculus batrachioides	0.17	100	1.02	1.04	1.01

Table 4. Contribution of species to floristic dissimilarity among the Control, 17.5g, 35 g, and 52.5g treatments based on SIMPER analysis (Bray–Curtis index)

Taxon	Contrib. %	Cumulative %	Mean 0 g	Mean 17.5 g	Mean 35 g	Mean 52.5 g
Isoetes velata	27.11	27.11	8.57	10.2	10.7	11.4
Ranunculus peltatus	14.72	41.83	4.63	7.02	7	4.07
Damasonium bourgaei	14.02	55.85	3.23	3.85	4.37	5.37
Eleocharis palustris	14.02	69.88	3.23	3.85	4.37	5.37
Callitriche truncata	10.05	79.92	2.78	4	3.83	2.92
Juncus bufonius	8.82	88.74	2.85	2.97	2.78	2.2
Callitriche brutia	5.79	94.54	1.88	2.33	2.37	1.77
Damasonium polysperum	2.81	97.35	1.37	1.35	1.48	1.57
Chara sp.	2.464	99.81	1.37	1.25	1.58	1.35
Ranunculus batrachioides	0.1882	100	1	1.02	1.05	1.03

bufonius, whose abundances all declined under the highest manure addition, from 4.00 to 2.92, from 2.37 to 1.77, and from 2.97 to 2.20, respectively (Table 4).

In contrast, *Isoetes velata*, *Damasonium bourgaei*, and *Eleocharis palustris* showed a continuous increase in abundance with increasing manure addition. These species-level contributions to dissimilarity suggest that the highest manure addition may have simplified the community in favour of a few tolerant taxa, at the expense of species with intermediate optima; however, this pattern is presented here as a compositional shift in response to organic enrichment rather than as evidence of eutrophication per se, since no trophic variable (nutrient concentrations, oxygen, algal biomass) was measured. Because manure addition had no significant effect on overall species richness, we no longer describe the low-to-intermediate additions as a 'diversity optimum'; rather, these results indicate a change in relative abundance and composition, without a demonstrated increase in richness.

DISCUSSION

The microcosm experiment revealed significant but unequal effects of hydrology and manure addition on the density and species richness of plant communities emerging from seed banks of Mediterranean temporary ponds. These two factors did not act at the same level: hydrology structured the overall community composition, whereas manure addition modulated, within this framework, the outcome of competitive interactions among species.

Effect of hydrology on germinated propagule density and species richness

As expected for this type of habitat, vegetation was largely dominated by annual species, particularly aquatic annuals, which accounted for nearly 90% of the community. This predominance reflects their strong adaptation to the fluctuating and stressful conditions characteristic of temporary ponds (Holland & Jain, 1981; Bliss & Zedler, 1998; Bauder, 2000;Grillas et al., 2004; Rhazi et al., 2005; Rhazi et al., 2006; El Madihi, 2020; Deil, 2020; Ben Bammou et al., 2021).

The hydrological regime differentiated functional groups in contrasting ways. Total germinated propagule density and species richness, as well as those of aquatic species, were significantly higher under the Wet (W) and Flooded (F) treatments, with no significant difference between them, suggesting a moisture threshold beyond which the additional benefit of prolonged inundation becomes marginal for these groups. Aquatic annuals, however, reached their highest values specifically under the Wet (W) treatment and remained low under both Dry (D) and Flooded (F) conditions. Their short life cycle therefore appears to be optimized under sufficient but non-permanent moisture, whereas prolonged inundation may limit oxygen availability or shorten the effective germination window. In contrast, aquatic perennials increased significantly with increasing inundation intensity, reaching their highest values under the Flooded (F) treatment, with no significant difference between Dry and Wet conditions. This response pattern, opposite to that of annuals, is consistent with their strategy of long-term persistence in aquatic habitats.

This contrast illustrates why hydrology is considered the main structuring factor of wetland communities, directly regulating ecosystem processes (Silvertown et al., 1999; Fraaije et al., 2015) and determining both vegetation composition and structure through germination, growth, and spatial distribution of plants along the flooding gradient (Euliss et al., 2004; Moor et al., 2017; Daniel & Rooney, 2021). The timing of inundation is as important as its duration: early inundation favors propagule production, whereas late spring inundation combined with a short hydroperiod depletes the seed bank without allowing its replenishment.

This dynamic helps explain the prevalence of temporary ponds in Mediterranean regions, where high interannual variability in hydrological regimes means that they should be viewed not as discrete habitats but as dynamic systems embedded within continuous environmental gradients, in which hydrological fluctuations shape regeneration pathways (Euliss et al., 2004). During summer and autumn droughts, biological activity becomes almost negligible, with most organisms persisting in the soil as seeds until the following season (Holland & Jain, 1981).

Hydroperiod length also acts as a filter of functional traits beyond species richness alone: long hydroperiods favor environmental stability and the development of larger organisms, whereas short hydroperiods impose stressful conditions that select for small-sized species with rapid reproduction (Solimini et al., 2005; Schriever et al., 2015). A reduction in hydroperiod therefore tends to decrease alpha and beta diversity (Ripley & Simovich, 2009; Cañedo-Argüelles et al., 2013; Fernández-Aláez et al., 2020), eliminating intolerant or slow-developing species in favor of traits associated with drought resistance, dormancy, and small body size (Schriever & Lytle, 2016).

The response of aquatic perennials observed here is consistent with the combination of morphological traits described as the "isoetid" type, which occurs in taxa characteristic of Mediterranean temporary ponds (*Isoetes* spp., *Juncus articulatus*, *Cyperus* spp.) and is interpreted as an example of evolutionary convergence in response to the hydrological constraints of these habitats.

These adaptations enable the associated hydrophytes to exploit wet conditions for enhanced growth and survival, notably resulting in greater plant height (Keeley & Zedler, 1998; Spencer & Rieseberg, 1998; Keeley, 1999; Deil, 2005). *Isoetes* particularly illustrates this deep-water strategy: in several isoetid species, abundance increases with water depth and inundation duration (Rhazi et al., 2009), while fluctuations in water level reduce competitive pressure from competing species (Gacia & Ballesteros, 1996).

Effect of manure on germinated propagule density and species richness

Total germinated propagule density, as well as the densities of aquatic species, aquatic annuals, and aquatic perennials, varied significantly according to the amount of manure added, with maximum values reached under the 17.5g and 35g treatments and no notable difference between them. Species richness, however, did not differ significantly among the four treatments. Thus, manure addition redistributed the relative abundance of species without necessarily excluding any species at the level of overall richness, clearly distinguishing its effect from that of hydrology.

This result may reflect a nonlinear response to enrichment, potentially consistent with a gradual shift toward competitive exclusion, although this mechanism was not directly measured in this study. Moderate nutrient addition could stimulate the biomass of most species (a possible fertilization effect), whereas at the highest dose, small and delicate annuals (the two *Callitriche species* and *Juncus bufonius*) declined while dominant species continued to increase. The significant increase in aquatic perennial density with increasing organic matter addition is consistent with this hypothesis: enrichment may favor species with a greater capacity to monopolize space and light, to the possible detriment of low-trophic-level specialists, although light competition and resource monopolization were not directly quantified here.

This interpretation is consistent with regional and international studies on the effects of organic enrichment in temporary ponds. In a mesocosm experiment on Moroccan temporary ponds, simulated trampling and sheep manure addition reduced plant and invertebrate richness following hatching (Van den Broeck et al., 2019). At a larger spatial and longer temporal scale, the effect can become destructive: eutrophication of the semi-permanent lake of Majen Chitane (Mogods, northern Tunisia), following cultivation of the adjacent peatland, resulted in the local extinction of 40–55% of characteristic hydrophyte species of temporary ponds, including species of

the Isoetion (Daoud-Bouattour et al., 2011). Similarly, in North American vernal-pool mesocosms, livestock excreta reduced plant diversity and could lead to the loss of macrophytes and functional decline of the ecosystem (Croel & Kneitel, 2011). At the landscape scale, plant community structure on both sides of the Strait of Gibraltar is related to nutrient loading (Lumbreras et al., 2016), while rare species with low tolerance to disturbance constitute good indicators of chemical and physical alterations of their environment (Rhazi et al., 2006).

These effects occur within a broader context: animal excreta threaten catchments, shallow water bodies, and ponds by promoting eutrophication and algal proliferation (Carpenter et al., 1998; Belsky et al., 1999; Smith et al., 1999; Anderson et al., 2002). The non-random distribution of manure and urine at the landscape scale probably exacerbates this pressure on temporary ponds, as livestock tend to concentrate around water points, thereby increasing the accumulation of excreta in and around ponds (Wallis de Vries & Schippers, 1994; Belsky et al., 1999). At the local scale, this enrichment in organic matter, nitrogen, and phosphorus creates favorable conditions for seedling establishment in naturally oligotrophic environments (Tilman, 1988; Matthews, 1992; Chapin et al., 1994). Deposition sites thus represent both a source of readily available nutrients and a potential source of propagules capable of accelerating colonization. However, this benefit has limits: at high livestock densities, the toxicity of fresh excreta may instead hinder vegetation establishment and development in the immediate vicinity of deposition sites (Raedeke, 1979).

The magnitude of this effect at realistic doses is well documented. High inputs of cattle excreta during the aquatic phase resulted in an 87% reduction in vegetation cover and a 54% decline in species richness during the subsequent terrestrial phase (Marty, 2015). This effect was mainly attributed to the development of algal mats under eutrophic conditions, which strongly reduce the amount of light available to vascular plants. A similar mechanism has been reported for litter effects in temporary ponds in southern France (Rhazi et al., 2004) and in terrestrial ecosystems (Ozimek et al., 1991; Zedler, 1987; Foster & Gross, 1998; Hautier et al., 2009). Other processes may also contribute, including competition for nutrients between algae and plants, changes in water quality (nitrate and ammonium), and interactions between water stress and nutrient enrichment (Van Donk & Van de Bund, 2002; Smith, 1982; Elser et al., 2007).

Combined effects of hydrology and manure on community composition

In Mediterranean temporary ponds, hydrology is the main factor structuring vegetation (Rhazi et al., 2001; Grillas et al., 2004; Sahib et al., 2009), with inundation duration controlling water availability, oxygenation, and soil organic matter mineralization, as well as the intensity of competitive interactions (Casanova & Brock, 2000; Nygaard & Ejrnæs, 2009). Long hydroperiods are therefore expected to favor aquatic and amphibious species, intermediate hydroperiods to allow the coexistence of the greatest number of species, and short hydroperiods to select for annual species with rapid life cycles (Bonis et al., 1995; Casanova & Brock, 2000; Brock et al., 2003; Grillas et al., 2004; Nygaard & Ejrnæs, 2009; Rhazi et al., 2009).

It is within this hydrological framework that the secondary but clear effect of nutrient enrichment operates. Manure addition enriches the environment with nitrogen, phosphorus, and organic matter (Rhazi et al., 2001; Bouahim et al., 2014), preferentially benefiting species with rapid growth and high biomass production (Grime, 1979; Keddy, 2000; Goodwillie et al., 2020). The SIMPER analysis clearly supports this trend and reveals, along this trophic gradient, a continuum of species-specific responses.

The abundance of *Isoetes velata* increased continuously along the manure-enrichment gradient. Although adapted to temporarily flooded soils (Grillas et al., 2004), this species may benefit from nutrient addition when water remains available for a sufficiently long period (Nygaard & Ejrnæs, 2009), progressively enhancing its competitive ability under the 35g and 52.5g treatments (Grime, 1979). Its dominance would therefore be expected under conditions combining long hydroperiods with moderate to high nutrient enrichment. *Damasonium bourgaei* and *Eleocharis palustris* showed a similar response: by rapidly exploiting nutrients released from manure (Grime, 1979), they are particularly favored as long as soil moisture remains sufficient (Nygaard & Ejrnæs, 2009), suggesting that their abundance should peak under intermediate to long hydroperiods combined with the 52.5g treatment.

In contrast, *Callitriche truncata* reached its optimum under the 17.5g and 35g treatments and subsequently declined under 52.5g. This unimodal response is consistent with the "hump-shaped" relationship between species richness (or abundance) and productivity (Keddy, 2000; Nygaard & Ejrnæs, 2009). It probably reflects an adaptation to low to moderately enriched waters, sensitivity to eutrophication (Bouahim et al., 2014), and increased competition with more vigorous species as fertility increases (Grime, 1979; Goodwillie et al., 2020). This species therefore appears to be characteristic of intermediate conditions, while *Callitriche brutia* follows a similar pattern, also appearing to favor moderate levels of enrichment (Nygaard & Ejrnæs, 2009).

Juncus bufonius, in contrast, showed a marked decline under the 52.5g treatment, suggesting an adaptation to relatively nutrient-poor substrates (Grime, 1979), low tolerance to competition induced by nutrient enrichment (Goodwillie et al., 2020), and progressive exclusion by more productive species (Keddy, 2000; Bouahim et al., 2010). It would therefore be expected to occur more frequently in control treatments, under short to intermediate hydroperiods, and in less fertile environments.

Finally, *Damasonium polyspermum*, *Chara* sp., and *Ranunculus batrachioides* showed only minor variations in abundance among treatments. Their distribution appears to be governed more strongly by hydrology than by trophic status (Rhazi et al., 2001; Sahib et al., 2009), which could explain their broad ecological amplitude (Grillas et al., 2004) and relative tolerance to variations in nutrient availability.

The significant PERMANOVA effect of hydrology should nevertheless be interpreted with caution: multivariate dispersions differed significantly among the three hydrological regimes (betadisper, $F = 53.51$; $P = 0.0001$), so part of this effect may reflect differences in within-group variability rather than differences in community centroids alone. In contrast, dispersions did not differ among the four manure levels ($P = 0.100$), which supports a centroid-shift interpretation of the manure effect on community composition.

Taken together, these results support the hierarchical model proposed in the Introduction: hydrology acts as the primary ecological filter, whereas manure addition acts as a secondary differentiating factor that favors the most competitive species within each hydrological regime (Rhazi et al., 2001; Nygaard & Ejrnæs, 2009; Bouahim et al., 2014). This pattern agrees with the observations of Rhazi et al. (2001), who found that vegetation in Moroccan temporary ponds was more strongly correlated with hydrology (water depth and inundation duration) than with trophic variables. Increasing manure additions therefore progressively promote the dominance of a small number of species with strong responses to nutrient enrichment, particularly *Isoetes velata*, *Damasonium bourgaei*, and *Eleocharis palustris*, at the expense of species adapted to oligotrophic or intermediate conditions, such as *Juncus bufonius* and the two *Callitriche* species, whose relative importance declines. This homogenization of communities and loss of specialist species under enrichment are widely documented in wetlands (Bouahim et al., 2014; Goodwillie et al., 2020) and suggest that, beyond a certain threshold, even moderate organic fertilization may compromise the conservation value of these priority habitats.

A limitation of this study is that the physicochemical characteristics of the manure (dry matter, organic matter, N, P, K, C/N ratio) were not measured. The manure treatments therefore cannot be related to specific nutrient loads, and the doses applied cannot be compared with nutrient-based thresholds reported in other studies; the responses observed are interpreted as effects of fresh manure mass rather than of N or P inputs. Future work should include laboratory analysis of the manure applied.

CONCLUSIONS

Mediterranean temporary ponds, particularly those in Morocco, are freshwater ecosystems rich in biodiversity but currently among the most threatened, owing to the combined effects of human pressures and climate change. This study aimed to determine how hydrology and manure addition influence the density, species richness, and composition of aquatic vegetation in these habitats.

The results show that hydrology is the main determinant of vegetation, significantly influencing the density of all species as well as species richness and community composition. Clear niche segregation emerged along the moisture gradient: *Isoetes velata*, associated with *Damasonium bourgaei* and *Eleocharis palustris*, dominated the most persistently flooded areas, whereas *Callitriche truncata* and *Ranunculus peltatus* developed under intermediate

conditions, and the pioneer species *Juncus bufonius* was restricted to the driest substrates.

The effect of manure was more nuanced: it altered community density and composition without significantly affecting overall species richness. Nutrient enrichment favoured tolerant species whose abundance increased with manure addition, at the expense of species with intermediate optima, which declined markedly at the highest addition rate. This compositional shift in response to organic enrichment shows that the highest manure addition altered the community rather than enriching it in species terms, without evidence of a distinct richness optimum at low to moderate manure additions. The significant interaction between hydrology and manure further indicates that the effect of nutrient enrichment depends on the hydrological context, while the positive correlation between density and species richness should be read in light of the partial mathematical dependency between these two measures noted above.

Overall, plant diversity in these temporary ponds depends primarily on the hydrological regime, while manure acts as a secondary factor, with a risk of compositional change and dominance by a few tolerant species when inputs become excessive. These findings highlight the vulnerability of these habitats to organic enrichment pressures and the need to preserve their natural hydrological functioning.

Future research should extend this work to species functional traits; compare the effects of animal manure, mineral fertilizers, and agricultural inputs transported by runoff in order to distinguish the effects of nutrients from those of organic matter; combine hydrological, manure, and trampling gradients to provide a more realistic assessment of cumulative pressures; validate the microcosm results through field observations; and strengthen conservation and sustainable management actions for these habitats.

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