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Differentiation of the Seed Regeneration Niche Along a Small-Scale Plant Zonation in Mediterranean Temporary Ponds

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ABSTRACT

Aims: Regeneration by seed is regarded as a key stage to understand plant community processes and vegetation patterns. In this study, we tested the hypothesis that the seed regeneration niche exhibits differentiation along the small-scale plant zonation that characterizes the ephemeral vegetation of Mediterranean temporary ponds (MTPs).

Location: MTPs of Sicily, Italy, southern Europe.

Methods: Seeds of 13 MTPs species were collected along the zonation. Experiments were conducted to measure seed germination responses to different levels of temperature (constant and alternating), light, and after-ripening. Cardinal temperatures for germination (base T_b, optimal T_d, ceiling T_c) were calculated following a thermal-time approach. Analyses were applied to (1) test germination responses as a function of the zonation and (2) describe the shape of the germination niche across zones.

Results: Germination temperatures significantly varied along the zonation, with species from the outer belt having higher T_d ($> 24.5°C$) and T_c ($> 30°C$) than species from flooded areas. Species from the long-lasting flooded area showed the highest germination in darkness ($> 75\%$ in some cases). Alternating temperatures and after-ripening had a general positive effect across zones. Ordination analysis indicated a high variability of light and alternating temperature preferences in species from short-lasting flooded areas.

Conclusions: Our results support the hypothesis that temperature and light preference for germination vary across a small-scale plant zonation in MTPs. This allows species from the outer belt to be among the first to germinate, whereas species from long-lasting flooded areas can germinate even under turbid water. The different light and alternating temperature preferences of species from short-term flooded areas may be an adaptation to cope with water fluctuations. This study confirms the importance of considering seed germination traits in the effort to understand the ecological dynamics of plant communities.

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1 | Introduction

Regeneration traits determine the colonization capacity of ephemeral herbs and the ecological niche in which they can successfully establish (Grubb 1977). Indeed, early stages of a plant's life cycle (germination and seedling establishment) are very sensitive to environmental factors such as temperature, light, and water availability (Longo et al. 2021). These and other factors act as "filters", selecting the species within a habitat according to the functional traits they possess (Keddy 1992). Nevertheless, efforts to understand plant community assembly are mainly focused on traits related to adult plants (such as vegetative growth, survival, and resource acquisition), and less attention is given to regeneration traits, including germination traits (Jiménez-Alfaro et al. 2016; Larson and Funk 2016). Accordingly, investigating and integrating seed traits into plant ecology is now identified as a priority to achieve a better understanding of ecological niches, population dynamics, and community assembly (Saatkamp et al. 2019). To confirm this, a growing number of studies are highlighting the key role of seed traits in explaining species occurrence and coexistence within communities (Poschlod et al. 2013; Fernández-Pascual et al. 2017; Tudela-Isanta et al. 2018; Rosbakh et al. 2020, 2022; Del Vecchio et al. 2021).

Wetlands have long attracted the scientific interest of vegetation and community ecologists because they are characterized by strong gradients of environmental factors such as temperature, light, and hydroperiod (duration, frequency, and depth of flooding), which vary in small spatial scales (across the habitat zonation) and across time (during seasonal changes in the water table). These properties make them an ideal "natural laboratory" to test trait-based theories (Moor et al. 2017). Especially in temporary wetlands, water and temperature fluctuations have stronger selective effects on plant communities than in other wetland types. In temporary wetlands, the aboveground plant cover is seasonal, and the habitat is water-saturated for only a part of the year (Deil 2005). Being ephemeral, small, endangered, and rich in specialist species, temporary pools represent a priority system for testing ecological theories of vegetation science (Blaustein and Schwartz 2001).

Mediterranean temporary ponds (MTPs) are shallow water bodies, heterogeneous in size, shape, depth, and elevation, typical of Mediterranean climates. Across the year, they are characterized by the alternation of a flooded phase, which starts with the autumn rains, followed by a dry phase, leading to the complete desiccation of the ponds during the Mediterranean dry summer season (Grillas et al. 2004). Being highly dependent on rainfall, MTPs are severely threatened by global change (Dimitriou et al. 2009; Grillas et al. 2021; Parra et al. 2021) and are considered a priority habitat for conservation in Europe (Council Directive 92/43/CEE, code 3170*). During the flooded phase, MTPs usually exhibit small-scale zonation, related to hydroperiod and the microtopography of the ponds: (1) long-lasting flooded areas (LL) in the deepest and central part of the ponds; (2) short-lasting flooded areas (SL) surrounding the LL; and (3) outer belts (OB) at the edge of the pond, which are never or seldom inundated (Caria et al. 2015). MTPs host a highly specialized flora which is distributed along this small-scale environmental zonation (Bauder 2000; Rocarpin et al. 2016; Lanfranco et al. 2020). In such stochastic habitats, trait responses to water fluctuations play a major role in the distribution of plant species along the zonation (Brock and Casanova 1997).

A common adaptive trait of MTPs plants is their annual life cycle, which enables them to survive summer drought in the soil seed bank (Médail et al. 1998). Building a large soil seed bank is a shared strategy to cope with unfavorable seasons or disturbance (Aponte et al. 2010). Although seeds play a key role in the survival and regeneration of MTPs' ephemeral vegetation, investigations about the plant ecology of this habitat have mainly focused on species composition (Minissale and Sciandrello 2016; Sciandrello et al. 2016; Lanfranco et al. 2020; Tomaselli et al. 2022). Only a few works have analyzed seed dormancy and germination in MTPs, generally focusing on species-level responses (Carta et al. 2012, 2013; Camilleri and Lanfranco 2013; Puglia et al. 2018; Di Stefano, Blandino, et al. 2025). A literature review by Carta (2016) proposed a common regeneration strategy of MTPs plants. This study indicated that the germination of these plants is generally characterized by the requirement for light and cool temperatures (≤15°C), and that dry after-ripening alleviates primary dormancy in many species (if present). However, such species-level studies provide limited insight into how regeneration traits scale up to shape communities across the microtopographic and hydrological gradients of MTPs. This lack of a community-level perspective leaves a gap in our understanding of how regeneration traits vary across the different environmental conditions that characterize the spatial gradients of this highly dynamic habitat.

Previous studies on the germination ecology of wetland plants outside the Mediterranean pointed out that species adapted to deep waters can germinate with high percentages in darkness, under constant temperatures, broadening their germination niche when physiological dormancy is broken (Rosbakh et al. 2020). In contrast, a preference for light was observed in species that experience stronger water fluctuations, typical of short-lasting flooded areas (Baskin et al. 1993; Tuckett et al. 2010; "true mudflat species" in Phartyal et al. 2020). These small-sized species also germinate better under fluctuating temperature regimes (Rosbakh et al. 2020), as this acts as a low-water level and an open-patch detection mechanism (Thompson et al. 1977). Furthermore, a preference for temperatures ≤20°C was found in MTPs species that colonize long-lasting and short-lasting flooded areas (Carta 2016). In the more terrestrial plant species that characterize the outer belts of wetlands, a broader germination niche width was observed, including high germination in darkness together with an insensitivity to temperature regimes (constant vs. alternating) ("facultative mudflat species" in Phartyal et al. 2020).

The main aim of this study was to analyze the germination traits of species inhabiting different zones of MTPs from a functional ecology perspective, to test the hypothesis that there is a differentiation of the seed regeneration niche along a small-scale plant zonation. In particular, we predicted the presence of specific germination adaptations to cope with the environmental variability along the small-scale zonation (Table 1). Using seeds of 13 MTPs specialist plant species, we conducted laboratory germination experiments to measure the seed germination niche of each species (concerning constant and alternating temperatures, light

TABLE 1 | Predicted optimal seed germination conditions across the MTPs small-scale plant zonation, based on studies conducted in other wetland habitats.

	MTPs small-scale plant zonation			
Environmental cues	**Long-lasting flooded area (LL)**	**Short-lasting flooded area (SL)**	**Outer belt (OB)**	**Ecological significance**
Temperature	≤20°C	≤20°C	Wide range	Perceiving seasonality, timing germination early or late in the rainy season (Finch-Savage and Leubner-Metzger 2006)
Alternating temperature	Constant	Alternating	Indifferent	Inhibit or stimulate germination under flooding (Thompson et al. 1977)
Light	High germination in darkness	High germination in light	High germination in darkness	Detecting seed position in the soil and the presence of water turbidity due to flooding (Bewley et al. 2013)
After-ripening	Positive effect	—	—	Delay germination in a season unfavorable for seedling survival

and after-ripening). Subsequently, we addressed two research objectives: (1) to test germination responses as a function of the zone; and (2) to describe the shape of the germination niche across zones.

2 | Methods

2.1 | Study System

For 2 years (from 2022 to 2024), floristic surveys were carried out during different seasons in several MTPs of Sicily, Italy (see Appendix S1 for details of the sites). Being the plant communities of MTPs characterized by seasonal successions (Bagella et al. 2009), and the different morphotypes changing over seasons (Fernández-Zamudio et al. 2021), surveys were carried out from autumn to summer to sample whole plant diversity. Additionally, in the different ponds, it was possible to recognize three microtopographic situations that characterize the MTPs' small-scale plant zonation (Figure 1). During the flowering season of each monitoring year, plant specimens were collected, and species were identified at the Germplasm Bank of the University of Catania (BGs-CT) following Pignatti et al. (2019). The identified species were classified in functional groups according to their morphological response to temporary flooding sensu Brock and Casanova (1997). Four functional groups of plants were identified: fluctuation-responder species with heterophylly; fluctuation-responder species with floating leaves; small-sized fluctuation-tolerator species; and terrestrial species of damp places. Species were then assigned to a zone according to their functional group and growth place observed during the surveys. Based on the information collected, 13 typical MTPs species occurring across the plant zonation of the surveyed ponds were selected (Figure 1 and Appendix S1).

2.2 | Seed Collection

Mature fruits or seeds (hereafter, "seeds") of the 13 selected species were collected at the time of natural seed dispersal (dates in Appendix S1) following international standards for wild seed collection (ENSCONET 2009). Maturity was assessed based on seed color (yellow or brown) and on the ease of detachment from the mother plant. Seeds were harvested from over 200 randomly selected plants. For *Callitriche brutia* and *Ranunculus sanicufolius*, due to their staggered dispersal that can start in water, it was necessary to collect all the mother plants together with the soil. Plants of these two species were then transferred to BGs-CT, where they completed their life cycle allowing for seed collection. To limit spatial pseudoreplication, seed collection of species from the same area involved different pond systems. Seeds were cleaned and germination tests were started within 2 weeks. Seed dry mass was measured as the mean of four replicates of 200 seeds (Appendix S1).

2.3 | Seed Germination Experiments

Germination experiments were performed following a factorial design using temperature (constant and alternating), light, and after-ripening as factors. The effect of temperature and temperature regime was studied by incubating seeds at seven constant temperatures (2°C, 5°C, 10°C, 15°C, 20°C, 25°C, 30°C) and four alternating temperatures (15/5°C, 20/10°C, 25/15°C, 30/20°C). For *Pulicaria*

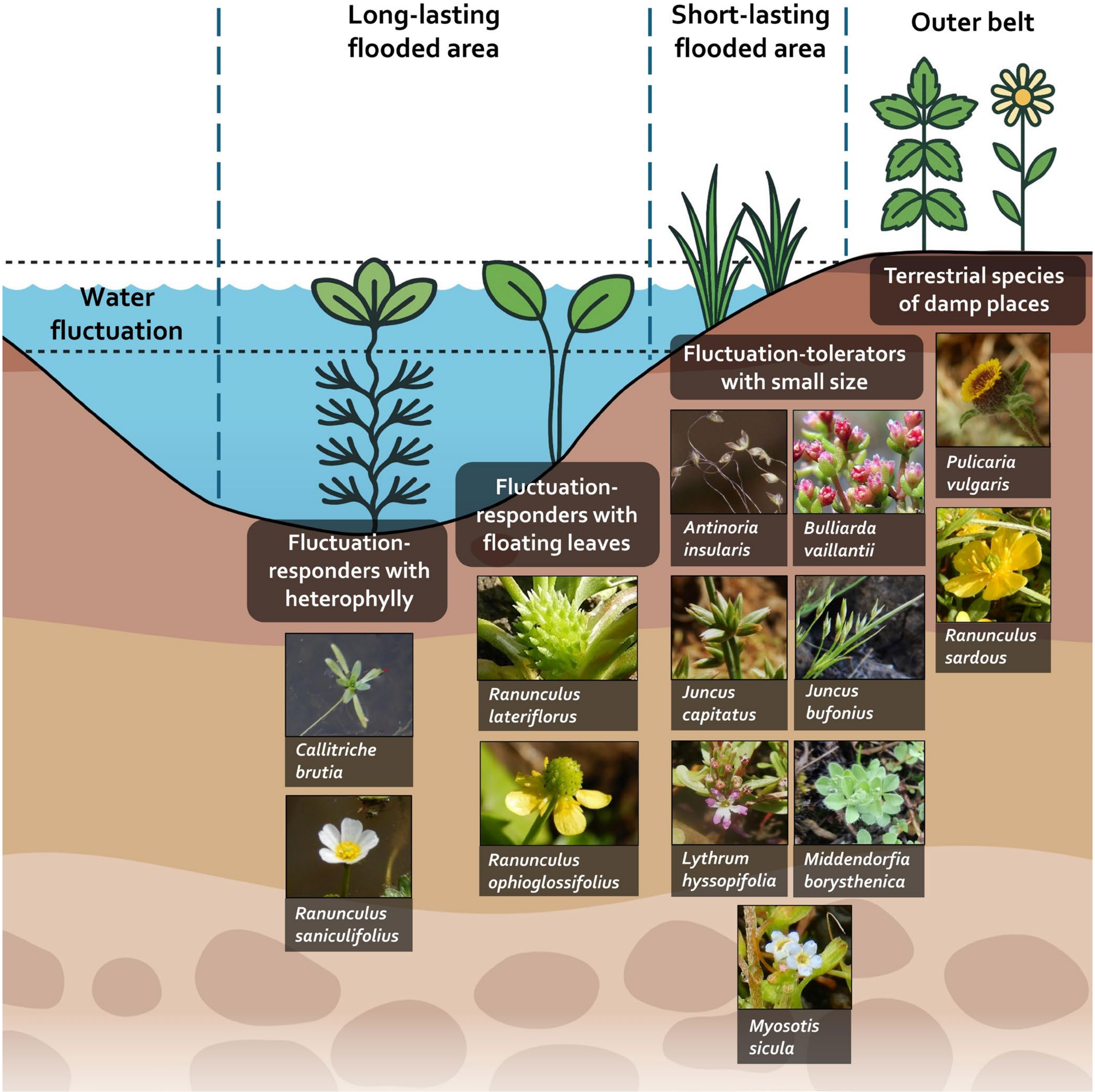

FIGURE 1 | Plant zonation and functional groups of plants of the surveyed MTPs. Seeds of 13 species were collected along the three areas that characterize the zonation: long-lasting flooded area, short-lasting flooded area, outer belt of the pond. Nomenclature followed Pignatti et al. (2019).

vulgaris, germination was also tested at 35°C due to the high and fast germination achieved at 30°C, even with fresh seeds. The results obtained for this species at 35°C were used only to calculate cardinal temperatures for germination (see below). Fluctuating temperature regimes were selected to mimic the temperature experienced by the seeds near the soil surface during the assumed germination season (autumn). Constant temperatures were used to simulate seed germination in flooded conditions, a situation in which the thermal oscillation between day and night is strongly buffered (Carta 2016; Rosbakh et al. 2020). To test the effect of light on germination response, each thermal condition was tested both in continuous darkness (D) and in alternating light/dark conditions (L/D) with a 12/12h photoperiod, simulating seeds being below or above the soil surface. In the alternating temperature regimes, the exposure to light coincided with the highest temperature. The effect of after-ripening on germination was evaluated by testing both fresh and after-ripened seeds. The after-ripening treatment was applied by storing dried seeds in darkness at 20°C in the presence of 50% relative humidity for 5 months.

Germination experiments were performed by sowing four batches of 25 seeds in 6 cm Petri dishes filled with 1% agar for each treatment (i.e., combination of constant and alternating temperatures, light, and after-ripening). For *C. brutia* and *R. saniculifolius*, two batches were used due to limited seed availability. Petri dishes were placed in germination chambers (MLR-351H; Sanyo—model. Tokyo, Japan), equipped with cool white fluorescent tubes (40 SS W/37; Osram FL, München, Germany).

For treatments in darkness, Petri dishes were wrapped in two layers of aluminum foil. Petri dishes were sealed with Parafilm M (Bemis Company Inc., Neenah, WI, USA) to prevent moisture loss. To ensure no effect due to the positioning within the growth chamber, Petri dish position was randomized every day.

Seeds incubated in light were counted daily, and germinated seeds were discarded, whereas dark-incubated seeds were counted only once at the end of the test to avoid any exposure to light. A seed was considered germinated when the radicle was longer than half the seed length. Germination tests lasted for 34 days, a week after the last germination was observed. At the end of the experiment, seed viability was evaluated by a cut test; seeds with a turgid and white embryo were considered viable. The final germination percentage (FGP) was calculated based on the total number of viable seeds. The percentages of nonviable seeds out of the total number of sown seeds were always ≤ 16.7%.

2.4 | Germination Responses as a Function of the Zone

We analyzed the data by fitting generalized mixed models with Bayesian estimation (Markov chain Monte Carlo generalized linear mixed models, MCMCglmms) using the R package MCMCglmm (Hadfield 2010), following the approach previously used in related studies (Carta et al. 2022). The final germination proportion of each germination test was the response variable in all the models (family = multinomial2). The fixed factors were experimental treatments, zone, and seed mass (scaled). A first model was fitted including the germination cues together with their interaction with zone and seed mass. A second model explored the main effect of zone and seed mass on germination proportion. The interaction between experimental treatments and zones provided a test of the first question, i.e., if MTPs' zonation shapes different germination niches. The reference level for the factor "zone" was set to "outer belt." The interaction between experimental treatments and seed mass was included to account for the potential effect of seed mass on germination responses and species ecologies. To account for the shared phylogeny of the study species, the models included as a random factor a reconstructed phylogenetic tree created by pruning Smith and Brown's (2018) updated mega-tree of the seed plants, using the R package U.PhyloMaker (Jin and Qian 2023). Weakly informative priors were used in the model, with parameter-expanded priors for the random effect. Models were run for 500,000 MCMC steps, with an initial burn-in phase of 50,000 and a thinning interval of 50, resulting, on average, in 9000 posterior distributions (de Villemereuil and Nakagawa 2014). From the resulting posterior distributions, we calculated mean parameter estimates and 95% highest posterior density and credible intervals (CIs). We interpreted the significance of model parameters by examining CIs, considering parameters with CIs overlapping with zero as nonsignificant.

2.5 | Shape of the Germination Niche Across Zones

To explore variation in the observed germination traits, that is, the shape of the germination niche, we performed an ordination analysis (principal components analysis; PCA), with species as objects and seed traits as variables. We considered the following seed traits: cardinal temperatures for germination, germination rate, the effect of constant and alternating temperature, light and after-ripening on germination, and seed mass. Cardinal temperatures for germination are defined as a set of three temperatures that describe the germination thermal window, as germination can take place only above a base temperature (T_b) and below a ceiling temperature (T_c), with the highest germination rate achieved at the optimal temperatures (T_d; Garcia-Huidobro et al. 1982). Cardinal temperatures for germination of after-ripened seeds were calculated following a thermal-time approach (Rowse and Finch-Savage 2003) using the "GRT.RFb" function of the "drcte" package in R (Onofri et al. 2022). To assess the role of temperature regime (constant and alternating), light, and after-ripening on seed germination, three odds ratios (ORs) were calculated: OR_{AT}, OR_L, and OR_{AR}. Germination rates (GR_{50}) were calculated under the best conditions, following the approach used by Fernández-Pascual et al. (2017). The calculation of the seed traits is described in detail in Appendix S2. Before performing the PCA, all variables were centered and scaled. All analyses in this article were performed in the R statistical environment (R Core Team 2025).

3 | Results

3.1 | Germination Responses as a Function of the Zone

FGPs of each species at each treatment are shown in Appendix S3. Across plant zones, species show a similar pattern of response to the germination treatments, generally preferring warmer and alternating temperatures, light, and after-ripening. However, there were differences between zones in the size of the responses. While species from LL and SL germinated well at cold to mild temperatures (between 5°C and 15°C), species from OB were able to germinate well also at warm temperatures (≥ 20°C). Alternating temperature regimes, light, and after-ripening generally stimulated the germination in species from all the zones, but species from LL germinated the most in darkness (Figure 2a).

This was confirmed by the MCMC models (Figure 2b): temperature, alternating temperature regime, light, and after-ripening all had a positive main effect on seed germination ($p < 0.001$, Appendix S4). But, compared with OB, the effect of temperature was negative in LL and SL (effect size of −0.356 and −0.344, respectively), which confirms the preference for colder temperatures in these species. Despite species from all zones germinating better under alternating regimes, a higher effect of alternating temperatures was found in species from LL ($p < 0.05$) compared to SL and OB ($p = 0.833$). Also, the interaction between light and zone highlights the fact that, despite light increasing germination in species from all zones, species from LL and SL showed a comparatively lower increase (i.e., a negative effect size) than in OB ($p < 0.001$ and $p < 0.05$, respectively). The positive effect of after-ripening was, on the other hand, similar across zones ($p = 0.478$ for LL and $p = 0.828$ for SL). Seed mass had a nonsignificant main effect on germination (Appendix S4); a nonsignificant interaction with alternating temperature ($p = 0.12$); a negative interaction with light and temperature ($p < 0.001$

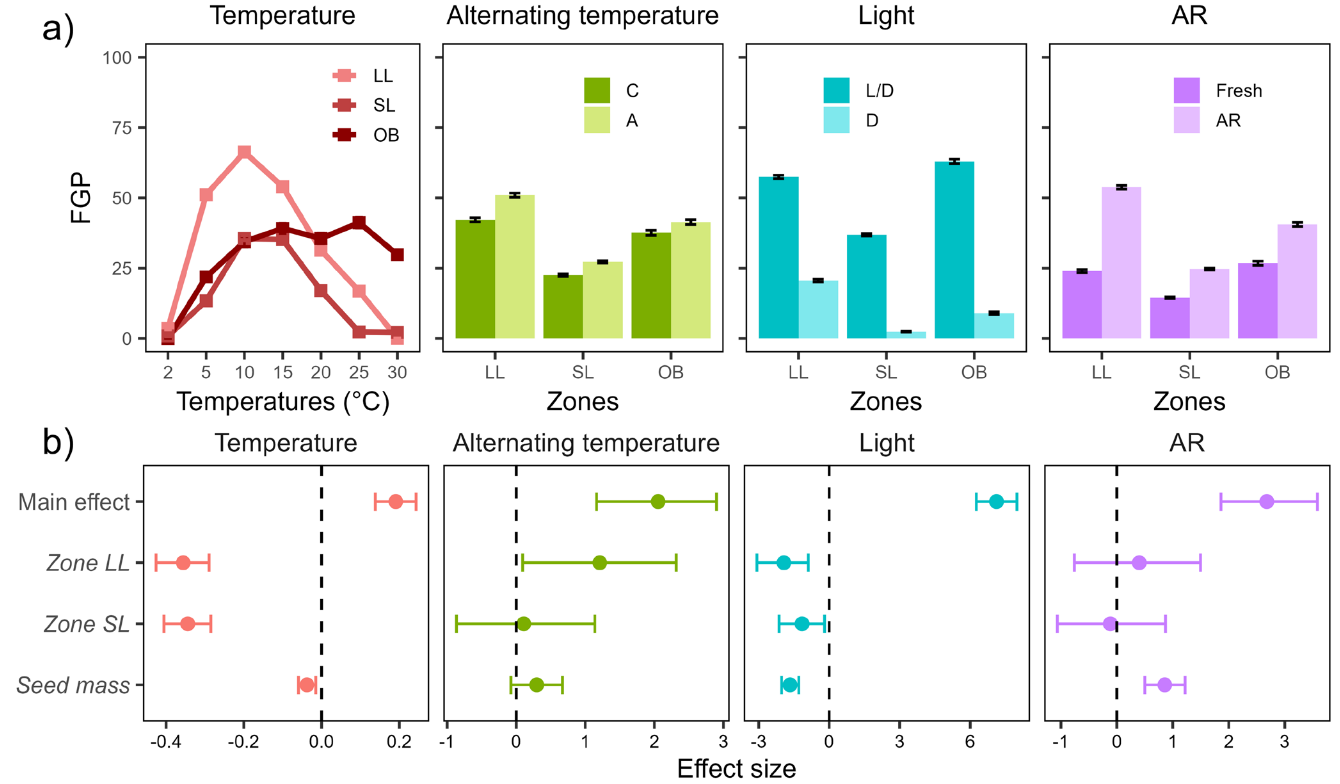

FIGURE 2 | Effect of different factors on the germination response of MTPs plants from different zones. (a) Final germination percentage (FGP ± SE) of species from each zone (LL: long-lasting flooded area; SL: short-lasting flooded area; OB: outer belt) at different constant temperatures (2°C, 5°C, 10°C, 15°C, 20°C, 25°C, 30°C) and under alternating temperature (C: constant; A: alternating), light (L/D: photo-period 12/12h; D: full darkness) and after-ripening (AR). (b) Main (regular font) and interaction effects (italic) of the fixed factors on germination proportion according to the MCMC models. Dots indicate the posterior mean of the effect size, and horizontal bars its 95% credible interval. Dashed vertical lines indicate the zero effect. For interactions with zones, OB (outer belt) was set as a reference. For example, significantly negative effect sizes in temperature for "Zone LL" and "Zone SL" mean that species from these two zones required significantly lower temperatures to germinate compared to OB.

in both cases); and a positive interaction with after-ripening ($p < 0.001$). This indicates that seed mass does not influence germination directly but instead shapes species' sensitivity to specific environmental cues: larger seeds tended to germinate better at lower temperatures, had a lower requirement for light, and a higher requirement for after-ripening. Species from LL generally germinated more than species from OB ($p = 0.02$), whereas no differences were found between germination in SL compared to OB ($p = 0.299$). An intermediate level of phylogenetic signal was found in the seed germination across zones, as pointed out by a Pagel's lambda (λ) value of 0.55, indicating an intermediate germination association with phylogeny.

3.2 | Shape of the Germination Niche Across Zones

The species–traits matrix used for PCA is present in Appendix S5, while germination rates and cardinal temperatures for germination of each species are present in Appendices S6–S8. The PCA was used solely as an exploratory visualization tool to explore variation in the shape of the germination niches, as statistical inference regarding species' responses was based on the fitted MCMC models. The first PCA axis explained 37% of the total variation in the seed germination niche (PC1, Figure 3). The quantitative variables with the largest contribution to this axis were T_c, T_d, GR_{50}, and OR_L, the last one with a negative correlation with the axis (Pearson's $r = -0.63$). This horizontal axis separated species with lower values of both optimal and ceiling temperatures and more sensitive to light, including five small-sized species from SL (*B. vaillantii*, *L. hyssopifolia*, *M. borystenicha*, *J. bufonius*, *J. capitatus*); from OB terrestrial species with higher optimal and ceiling temperatures (*P. vulgaris* and *R. sardous*). Intermediate between these species were the four species from LL (*C. brutia*, *R. lateriflorus*, *R. ophioglossifolius*, and *R. saniculifolius*) that showed higher T_d and T_c than species from SL but lower than OB. The second PCA axis (PC2, Figure 3) explained 30% of the total variation. The main contribution to this axis was given by T_b, OR_{AT}, and seed mass, with the first positively correlated with the axis (Pearson's $r = 0.82$). So, this axis separated species that possess a clear preference for alternating temperature, with bigger seeds and lower T_b (negative values of PC2, Figure 3), from those species with smaller seeds, insensible to alternating temperatures, and with higher T_b (positive values of PC2, Figure 3). Moreover, this axis separated species from SL in two groups: species with higher germination in alternating regimes (*Antinoria insularis* and *Myosotis sicula*) that showed negative values for this axis, and species insensitive to alternating temperatures (*L. hyssopifolia*, *M. borystenicha*, *J. bufonius*, *J. capitatus*) or with a preference for constant temperatures (*B. vaillantii*) that, on the other hand, had positive values (Figure 3). Overall, the broad dispersion of SL species along both PCA axes indicates that this zone encompasses a wide range of

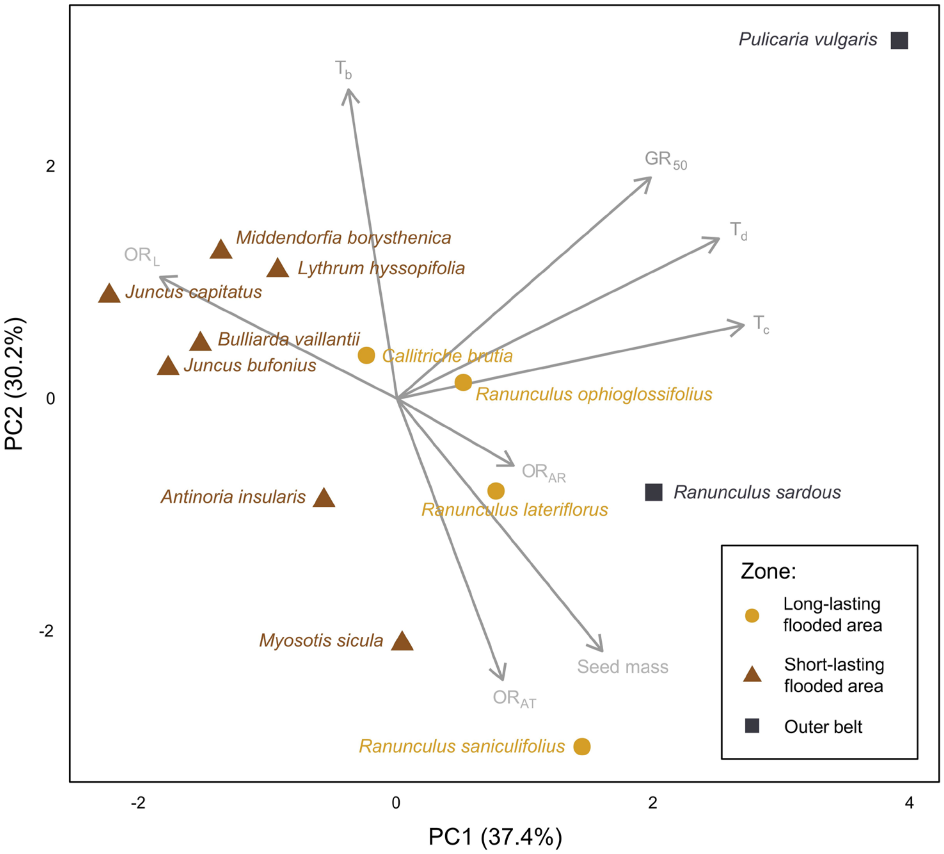

FIGURE 3 | PCA of the species–traits matrix of the 13 studied MTPs species across the small-scale plant zonation. Arrows indicate the direction and contribution of each trait to the axes, and these have been multiplied by 5 for easier visualization. T_b, T_d, and T_c are the cardinal temperatures for germination of after-ripened seeds, calculated according to equation 1 of Appendix S2. OR_{AT}, OR_L, and OR_{AR} are the odds ratios for the effect of alternating temperature, light, and after-ripening, respectively, on germination, calculated according to equations 2–4 of Appendix S2. GR_{50} is the germination rate for the 50th percentile at the optimal condition.

germination strategies, mainly in relation to response to light (PCA1) and preference for alternating temperature (PCA2).

4 | Discussion

Our study confirmed the hypothesis that there is a differentiation of the seed regeneration niche along the small-scale plant zonation of MTPs. This differentiation confirms some of our initial predictions (Table 1). In particular, species from flooded areas (both long-lasting and short-lasting flooded areas) generally germinated at lower temperatures and had lower base and ceiling germination temperatures than species from drier areas (outer belts). This highlights an adaptation of flood-adapted species to germinate late in the winter rainy season, when temperatures are colder and precipitation is well established. In contrast, species of the outer belt clearly prefer higher temperatures for germination (Table 2). This preference ensures that these species are ready to germinate at the end of summer, soon after the first rains start. This feature gives an advantage to the fast colonization of vacant spots in the outer belts of MTPs (Bonis et al. 1996). In this sense, being among the first to germinate also gives a chance to colonize the most depressed areas of the ponds in years in which these are poorly flooded, as it is observed during dry years (Fernández-Zamudio et al. 2018; Caria et al. 2021). In contrast, when rain fills the pond, flooding will filter these species out of the long-lasting and short-lasting flooded areas (Casanova and Brock 2000). In temporary ponds under a Mediterranean climate, flooding indeed allows amphibious species to escape the competition of the more generalist terrestrial species (Bliss and Zedler 1997).

Regarding temperature preference, it should also be noted that species from the short-lasting flooded area generally had the highest base and lowest ceiling temperatures, resulting in a narrow temperature range for germination, highlighting the specialization of their regeneration niche. Due to their small size and rapid life cycle, germination of short-lasting flooded area species is hypothetically possible in two temporal windows: during autumn, when the ponds are filling, and in spring, when the water recedes. Whether the triggering of a secondary dormancy could prevent spring germination through dormancy cycling has yet to be studied (Batlla and Benech-Arnold 2010; Finch-Savage and Footitt 2017). In contrast, the higher ceiling and optimal temperatures of species from the long-lasting flooded area compared to the short-lasting flooded area suggest a wider temperature range and regeneration niche. But it should be noted that this wide temperature range was only achieved after the release of physiological dormancy by after-ripening. After-ripening not only resulted in a forward shift of those two cardinal temperatures but also led to a general increase in germination rates. This can represent an adaptation to avoid

TABLE 2 | Optimal germination conditions found across the MTPs small-scale plant zonation and ecological conclusions.

	MTPs small-scale plant zonation			
Environmental cues	**Long-lasting flooded area (LL)**	**Short-lasting flooded area (SL)**	**Outer belt (OB)**	**Ecological conclusion**
Temperature	≤20°C	≤20°C	Wide range	Main correlation with plant zonation; perceiving the rainy season
Alternating temperature	Alternating	Species-specific	Alternating	Detecting the water table
Light	Germination possible in darkness	Species-specific	Light stimulated	Sensing turbidity of the water and seed position in the soil; highly dependent on seed mass
After-ripening	Positive effect	Positive effect	Positive effect	Common adaptation to the summer drying of the habitat

out-of-season germination: in years with longer hydroperiods, these species can indeed disperse during the flooded phase of the habitat (Fernández-Zamudio et al. 2021), and a low ceiling temperature in fresh seeds would prevent them from germinating too soon after dispersal (and therefore be exposed to summer drought). On the other hand, the temperature range for germination could be wider than observed if a residual dormancy sensu Batlla and Benech-Arnold (2015) persists following after-ripening. Conversely, in seeds only weakly affected by after-ripening, physiological dormancy could (1) not be present or (2) may still remain, requiring other pretreatments to be broken, such as warm or cold stratification. In this latter case, however, our results indicate that some seeds would nonetheless germinate rapidly at the end of summer, if precipitation arrives and soil temperatures fall within the species-specific thermal window for germination. Light significantly stimulated germination in species across the zonation. Despite this, species from the long-lasting flooded area showed the highest germination in darkness, confirming the prediction that species from deeper water are less sensitive to light. Also, in the pond specialist *Ranunculus baudotii*, a fluctuation-responder species with heterophylly that colonizes the long-lasting flooded area, high germination in darkness was scored (Carta et al. 2012). Light response was furthermore negatively correlated with seed mass, as these two traits may have co-evolved (Milberg et al. 2000). The adaptation to germinate in darkness ensures that seedling emergence can occur even under flooding or in the presence of water turbidity in wetland species (Baskin and Baskin 2014). In this sense, a recent study confirms that germination in darkness is possible even under hypoxia in several species from MTPs, including the ones inhabiting the long-lasting flooded areas (Di Stefano, Dominguez, et al. 2025). But the germination response of MTPs species to biogenic ethylene, typically produced by decomposed wetland sediment in flooded conditions (Phartyal et al. 2022), needs to be investigated. Nevertheless, this trait, together with a wide temperature range for germination, widens even more the germination niches of species from the long-lasting flooded area, after the release of physiological dormancy with summer after-ripening. A broad germination niche was also observed by Rosbakh et al. (2020) in species from deep waters, and this was interpreted as an opportunistic behavior that allows species to take advantage of early establishment. Considering the high unpredictability of precipitation in the Mediterranean area, these germination traits can represent useful tools to cope with the high interannual environmental variability typical of MTPs.

Alternating temperatures and after-ripening had a general positive effect throughout the zonation. This rejects the prediction that in MTPs, the preference for a specific temperature regime (constant or alternating) can change from the center to the edge of the ponds (Table 1). Furthermore, the preference for alternating temperatures is unrelated to seed mass, as seen in other European species (Carta et al. 2022), confirming that this trait has a poor explanatory ability when taken alone (Larson and Funk 2016). Despite the general enhancing effect of after-ripening, in the short-lasting flooded zone coexist species with a clear preference for alternating regimes (*A. insularis* and *M. sicula*), constant temperatures (*B. vaillantii*), and, on the other hand, species insensitive to alternating temperatures (*L. hyssopifolia*, *M. borystenicha*, *J. bufonius*, and *J. capitatus*). The preference of *B. vaillantii* germination for constant temperatures was also confirmed by Carta et al. (2013). These different temperature regime preferences can be an adaptation to water fluctuations, which are stronger here than in the other zones. The high environmental variability in terms of light and temperature regime typical of this zone could have indeed selected different sets of germination traits. In the same line, the response to light at the species level also varied in this zone, as the germination of *A. insularis* and *M. sicula* was possible in darkness, whereas light was a strict requirement for the germination of the other species from the short-lasting flooded area. Moreover, the common positive effect of after-ripening, together with the preference for alternating regimes, can be interpreted as a common adaptation to cope with the same two abiotic stresses that characterize MTPs: the extreme summer drying and the subsequent severe autumn flooding. During the dry months after the dissemination in late spring, seeds can indeed experience a general increase in germination rate across zones and, in some species, a notable forward shift in ceiling temperatures, as pointed out by the thermal time models. At the onset of precipitation, the seeds are thus ready to germinate rapidly and with high percentages, especially near the soil, where fluctuating temperatures are perceived. The general preference for light, furthermore, allows ungerminated seeds above the soil surface to build up a large seed soil bank. This was identified as a common adaptation to overcome periods with unfavorable weather conditions, allowing the long-term survival of the MTPs plant communities (Aponte et al. 2010).

Climate change is expected to substantially reshape the hydrological and thermal regimes of MTPs (Dimitriou et al. 2009; Grillas et al. 2021; Parra et al. 2021), with possible consequences for the recruitment strategies of annual wetland plants. Our results indicated that the three areas of the MTPs plant zonation differ in the shape of their germination niches, which may translate into contrasting levels of resilience. Species from the outer belt, which prefer higher temperatures for germination, may be able to exploit occasional summer rainfall events and maintain recruitment even under more erratic precipitation regimes and soil temperature increase, both consequences of climate change. Conversely, the short-lasting flooded areas host species with the widest diversity of germination responses, suggesting an ecological strategy that may buffer erratic precipitation patterns. Furthermore, the ability of these short-sized species to rapidly complete their life cycle could assure seed dispersal even in case of premature soil desiccation, due to out-of-season drought. Finally, species from the long-lasting flooded zone acquire, following after-ripening, a wide germination range, which may allow them to germinate rapidly once suitable conditions eventually occur, even under delayed autumn–winter flooding. Together, these patterns highlight that germination traits may contribute to differential climate change resilience across belts. But they also underline the need for future studies on different stages of the regeneration niche (Grubb 1977), such as seedling establishment and early survival. These studies would increase our knowledge of the impact of climate change on the annual regeneration of MTPs plant communities.

5 | Conclusion

In conclusion, this study (1) confirmed the hypothesis that germination preference for temperature and light varies across a small-scale vegetation zonation in MTPs; (2) refuted the hypothesis that species from the long-lasting flooded area germinate better at constant temperatures; and (3) pointed out that a general positive effect of after-ripening can be interpreted as a common adaptation to the habitat (Tables 1 and 2). These results can help practitioners to develop conservation and restoration programs in these highly threatened habitats, applying seed-based actions that are based on a sound knowledge of the species' seed biology (Ladouceur et al. 2018). In particular, sowing after-ripened seeds before the onset of the rainy season, ensuring topsoil movements that favor light-requiring species reaching the surface, and using the germination data of this study to develop propagation protocols may improve the success of conservation and restoration actions. Results support the role of the germination niche to explain species occurrence and coexistence in stochastic habitats (Grubb 1977), highlighting the importance of taking into account seed germination traits in the effort to understand the ecological dynamics of plant communities (Jiménez-Alfaro et al. 2016; Saatkamp et al. 2019). Although these results allowed us to define the germination adaptations of MTPs plant species along the zonation, future research should formulate and test explicit hypotheses linking germination traits of small species from short-lasting flooded areas to microenvironmental variation. By combining seed-trait databases (Fernández-Pascual et al. 2023; Cruz-Tejada et al. 2024) with in situ measurements (soil moisture, temperature, and flooding duration), we would have a more mechanistic understanding of how the contrasting germination niches found in these species correspond to distinct microtopographic or hydrological conditions.

Author Contributions

M.D.S., G.D.P., C.B., and A.C. conceived the study. M.D.S. performed the seed germination experiments. M.D.S. and E.F.-P. analyzed the data. M.D.S. led the writing process. G.D.P. and A.C. supervised the research. A.C. acquired the funds. All authors helped in critically revising the manuscript and gave final approval for publication.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that supports the findings of this study are available in the supporting information of this article. Raw germination data are available on request from the corresponding author.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Appendix S1:** List of the 13 selected and collected species. **Appendix S2:** Seed trait calculation. **Appendix S3:** Final germination percentages (FGP, ± SE) of the 13 studied species from three different zones of MTPs. **Appendix S4:** Summary of the phylogenetic generalized mixed models with Bayesian estimation (MCMCglmms). **Appendix S5:** Matrix of the seed traits of the 13 studied species. **Appendix S6:** Germination rates at constant temperatures and fitted thermal-time models of the 13 studied species. **Appendix S7:** Germination rates at alternating temperatures of the 13 studied species. **Appendix S8:** Cardinal temperatures for germination of the 13 studied species.