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Original Article

Influence of storage and temperature on the germination of a macrophyte seed bank from a temporary pond in the Brazilian semiarid

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ABSTRACT

Seed bank (SB) formation in temporary ponds is key to the local resilience of macrophyte species after drought disturbance. Studying seed germinability and its response to temperature is crucial to understanding regeneration strategies in wetlands, where seed banks play a central role. The objective of this work was to evaluate, through two experiments, the effect of storage period and temperature on the germination of the macrophyte seed bank from a temporary pond in the Brazilian semiarid region. In the first experiment, germinability was compared between fresh samples and samples stored in a cold chamber. In the second experiment, the effect of temperature on germination was evaluated using stored sediment. The fresh SB showed higher richness and lower seedling density than the stored sample. The higher temperature treatment (37 °C) led to higher richness and seedling density. *Lemna* sp. and *Heteranthera limosa* were the only species that germinated and emerged from the SB under all tested temperature conditions. The study concluded that cold storage can aid the conservation of temporary pond seed banks and that, within the temperature range, seed bank germinability was maintained under elevated temperatures representative of projected climate scenarios.

Keywords: Caatinga, ex situ conservation, resilience seeds, seed germinability, temporary wetlands.

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Introduction

Aquatic habitats subjected to cycles of flooding and drought are defined as temporary ecosystems and are often classified as biodiversity hotspots due to their unique ecological dynamics (Lukács *et al*., 2013; Calhoun *et al*., 2017; Parra *et al*., 2021). Temporary habitats are distributed globally, and are especially numerous in regions with highly seasonal climates, such as semiarid drylands (Lumbreras *et al*., 2016; Albuquerque *et al*., 2020). The temporary ponds in drylands formed during rainy seasons are shallow and isolated from any terrestrial water source, and usually evaporate completely during the dry season (Tabosa *et al*., 2012; Carta, 2016). Despite their ecological importance, temporary ponds are increasingly threatened by human pressures such as land-use change, agricultural intensification, water extraction, and pollution (Bagella *et al.,* 2016).

In Brazil, temporary ponds are common in the semiarid northeastern region, where the soil is typically shallow, and rainfall is low, with a long dry season, spanning from 8 to 10 months of the year (Maltchik, 2000). The plants in these ponds are mainly macrophytes, which are aquatic plants visible to the naked eye that grow fully or partially submerged in aquatic environments (Esteves, 2011). These species rely on rapid life cycles to develop and release seeds/propagules before the end of the rainy season (Esteves, 2011; Tabosa *et al*., 2012). The accumulation of desiccation-resistant propagules, such as seeds and spores of macrophytes, in the dried sediment of temporary ponds contributes to the formation of a soil seed bank (Ferreira & Catian, 2016).

Propagule banks are natural reserves of viable reproductive units found in the soil, including seeds and spores, capable of germinating and forming new plants when environmental conditions are favorable for germination (Douh *et al*., 2018). These propagules play an essential role in vegetation regeneration, allowing species persistence during adverse periods until conditions permit germination and reestablishment of aquatic vegetation (Fernández-Zamudio *et al*., 2018). Thus, they contribute to the maintenance of biodiversity, facilitate the recolonization of disturbed habitats, and support the functional stability of aquatic ecosystems (Bonis *et al*., 1995; Duarte *et al*., 2020).

Seed banks consist of transient seeds, which remain viable in the soil for up to one year, and persistent seeds, which survive for many years by enduring multiple wet-dry cycles, allowing germination to occur over extended periods (Vandvik *et al*., 2016; Baskin *et al*., 2019; Wang *et al*., 2020). Certain macrophyte species produce orthodox seeds, characterized by their ability to tolerate desiccation and low temperatures, thereby facilitating their storage and long-term viability in *ex situ* conservation (Hay *et al*., 2000; Tuckett *et al*., 2010; Carta *et al*., 2013). In the case of temporary pond sediment in drylands, these seed banks are predominantly formed by persistent seeds, and storing sediment samples dry at room temperature is often sufficient to preserve their viability (Brock, 2011).

Semiarid regions are expected to be disproportionately affected by climate change, characterized by rising temperatures, reduced rainfall, and decreased soil moisture (Scholes, 2020). These changes intensify evaporation and accelerate the desiccation of aquatic environments, including temporary ponds (Marengo *et al*., 2017; Ouhamdouch & Bahir, 2017; Xia *et al*., 2017). Such alterations threaten the persistence of macrophyte populations and their associated soil seed banks, which are essential for long-term ecosystem resilience. The reduction in seed bank density not only compromises the regenerative capacity of these systems but also heightens the extinction risk for species dependent on temporary aquatic habitats (Faist & Collinge, 2015; Calhoun *et al*., 2017; Grillas *et al*., 2021).

In addition to reductions in seed bank density, climate change may also impact seed viability and germination behavior. Extreme temperatures, both high and low, can inhibit germination of various species (Tuckett *et al*., 2010). Exposure to low temperatures can reduce enzymatic activity and metabolism in seeds, which may result in the inhibition of the germination process (Liu *et al*., 2022). Subsequently, rising global temperatures may drastically alter seed behavior within soil seed banks, disrupting the balance between quiescent and dormant states, or between germination and seedling survival following rainfall events (Walck *et al*., 2011; Ooi, 2012). For example, seeds may germinate in response to brief rainfall, but insufficient soil moisture may lead to high seedling mortality. (Ooi *et al*., 2009; Akinyemi *et al*., 2019). Investigating the composition and ecological dynamics of seed banks in temporary ponds is therefore essential for understanding the ecological history and resilience of these unique habitats. Furthermore, the evaluation of the seeds contained in seed banks provides insights into their long-term viability and supports the development of ex situ conservation strategies.

Therefore, this study aimed to evaluate the germination capacity of aquatic macrophyte seed banks after three and four years of cold storage and their ecological response to varying temperatures. Specifically, the objectives were: i) to evaluate whether seed banks composed of macrophyte propagules germinate after storage for three years in a cold chamber; and ii) to evaluate the ecological response of these seed banks after four years of storage when subjected to different temperatures (17 °C and 37 °C). Our hypotheses are: i) the macrophyte seed bank maintains germinability after at least three years of storage under cold-chamber conditions (10 ± 2 °C and 20% RH); and ii) seedling emergence responses differ among the temperature regimes tested.

Materials and Methods

Study Area

The study was carried out in a temporary pond undergoing eutrophication, located in the municipality of Sobradinho (9°27'29" S, 40°48'29" W, elevation 371 m), state of Bahia, Brazil. The pond has an area of 0.175 ha, a perimeter of 239 m, a maximum water depth of 0.6 m, and a volume of 370 m^3. This temporary pond was chosen for the study, among other accessible ponds, because it showed a seed bank with high density and species richness of macrophytes (Pereira, 2017). The local biome is classified as a seasonally dry tropical forest in the Caatinga phytogeographic domain (Pennington *et al*., 2000). According to the Köppen scale, the climate in the region is BSh (hot semiarid), with high temperatures, long dry periods, and irregular rainfall, concentrated from December to March. Average annual temperature and rainfall are 27 °C and 800 mm, respectively (Alvares *et al*., 2013; Pinheiro *et al*., 2020).

Seed bank (SB) sampling

In September 2014, during the dry phase of the temporary pond, when the substrate was completely dry under natural conditions, we collected 20 sediment samples from the bed of the dried temporary pond within a 250 m^2 sampling plot located in the undisturbed deepest region of the lagoon, avoiding the margins that had been traversed by animals and people. The samples were randomly collected within this plot using a post hole digger (0.11 m diameter) at a depth of up to 0.1 m, resulting in a sampled volume of approximately 9.51×10^{-4} m^3 per core (corresponding to an area of 0.044404 m^2). Each one of the sediment samples was then sieved and homogenized after removal of pebbles and rhizomes. Immediately after collection, part of the samples was used to evaluate seedling emergence from the fresh seed bank (FSB). The remaining samples were stored in sealed plastic bags under controlled cold conditions (10 ± 2 °C and 20% relative humidity) and later used in two independent experiments: one conducted after three years of cold storage, in September 2017 (Experiment 1), and another conducted after four years of storage under cold conditions (10 ± 2 °C and 20% relative humidity), in September 2018 (Experiment 2). Each experiment addressed distinct objectives and was analyzed independently.

Evaluation of the seed banks

Fresh and/or stored samples were assessed in two independent experiments, as described below. In both experiments, germination was defined as seedling emergence, and seed bank composition was assessed based on all seedlings that emerged during a six-week monitoring period, following the criteria of Gross (1990). Each of the 20 samples was considered an experimental unit (~replicate). Each experimental unit consisted of 150 mL of pond sediment, measured using a graduated beaker, mixed with 150 mL of expanded low-density vermiculite. The mixture was placed in individual rectangular aluminum trays (203 × 133 mm, 4.2 cm depth), evenly distributed to form a 3 cm-deep layer, and covered with a 1 cm layer of tap water.

Vermiculite was mixed with the sediment to improve substrate aeration, prevent compaction during flooding, and facilitate uniform seedling emergence. Control trays containing only vermiculite and the same water layer were randomly distributed among the experimental units to confirm that all emerged seedlings originated from the sediment samples. Each experiment lasted 42 days (six weeks). Seedling emergence was monitored weekly, and to prevent feedback sowing, all emerged seedlings were removed every 15 days, resulting in three removal events during the experimental period.

The emerged plants were identified at the genus or species level, following Bueno *et al*. (2011), Souza & Lorenzi (2012), and Flora and Funga of Brasil (2022). When diagnostic morphological characters were insufficient for species-level identification at early developmental stages, individuals were recorded at the genus level (e.g., *Ludwigia* sp.). The density was estimated using the seedling emergence method proposed by Van Der Valk & Davis (1978), in which viable propagules are quantified through seedling emergence under controlled conditions. Values were standardized and expressed as seedlings per square meter (seedlings m^{-2}) based on the sampled sediment surface area. The species richness (S) was determined according to the number of species germinated.

Experiment 1. Effect of seed bank storage on the emergence of seedlings

In Experiment 1, seedling emergence from the fresh seed bank (FSB) was evaluated shortly after sample collection in September 2014. The same sediment samples were then stored for three years in the dark in a cold chamber (Model RLC-050-B, MC Quay, São José dos Campos, Brazil) at 10 ± 2 °C and 20% relative humidity, yielding the stored seed bank (SSB). Seedling emergence from FSB (2014) and SSB (2017) was assessed under comparable environmental conditions in a polyethylene-covered greenhouse with 50% shading, during the same season, at an average temperature of 27 ± 2 °C and a 12-hour photoperiod. These assessments were compared in terms of seedling density and species composition.

Experiment 2. Effect of temperature on the emergence of seedlings

In Experiment 2, seed bank samples stored for four years in a cold chamber at 10 ± 2 °C and 20% relative humidity (from September 2014 to September 2018) were assessed under contrasting temperature conditions representing the minimum and maximum average temperatures recorded at the collection site (Embrapa, 2025). Seedling emergence and seed bank composition were evaluated either in a controlled germination room at a constant temperature of 17 °C (SB17) or in a glass greenhouse under natural light and naturally fluctuating temperatures, with a mean ambient temperature of approximately 37 °C (SB37).

Statistical analysis

Before analysis, data from Experiment 1 were evaluated for normality using the Shapiro–Wilk test at a 5% significance level. The residuals met the assumptions of normality ($p > 0.05$), allowing the use of parametric statistics. Therefore, differences in mean seedling density between the fresh seed bank (FSB) and the stored seed bank (SSB) were assessed using an independent-samples t-test.

To analyze the effect of temperature on seedling emergence from the SB17 and SB37 samples, we applied generalized linear modeling (GLM) to fit the data, after confirming data residuals with non-normal distribution ($W = 0.764$; $p < 0.001$) and non-homogeneous variances ($L = 27.855$; $p < 0.001$) (Shapiro & Wilk, 1965; Levene, 1960). Therefore, we chose not to use the angular transformation for the response variable. (Silva *et al*., 2022a). The distribution and link function were the quasibinomial distribution with the logit link function. After the GLM, the significant differences between temperatures were evaluated by pairwise comparison of the means by the post-hoc Tukey test at 5% significance. The means followed by the standard errors of the mean were fitted by the Šidák method (Šidák, 1967). All the statistical procedures were carried out with the R software (R Core Team, 2022). The following R packages were used to carry out the analyses: *car* (Fox & Weisberg, 2019), *fBasics* (Wuertz *et al.*, 2023), *nortest* (Gross & Ligges, 2015), *boot* (Canty, 2002), *emmeans* (Lenth, 2024), *multcomp* (Hothorn *et al.*, 2008), and *multcompView* (Graves *et al.*, 2023).

Results

Experiment 1. Effect of seed bank storage on the emergence of seedlings

A total of 15 taxa were recorded in the fresh seed bank (FSB), whereas seven taxa emerged from the stored seed bank (SSB) after three years of cold storage (Table 1). The species identified belonged to ten families, with Onagraceae and Poaceae showing the highest richness in FSB, while Araceae was the most representative family in SSB.

The FSB comprised a wide range of biological forms, including emergent, amphibious, free-floating, fixed-floating, and submerged species. In contrast, the SSB was mainly composed of free-floating and emergent species, indicating a shift in seed bank composition after storage (Table 1).

In terms of density, the most abundant taxa in the FSB were *Azolla* sp. and *Heteranthera limosa*, whereas in the SSB, *Azolla* sp. and *Lemna* sp. showed the highest emergence values (Table 1). These results indicate changes not only in species richness but also in the dominance structure of the seed bank after storage.

Mean total seedling density did not differ significantly between the stored seed bank (SSB) and the fresh seed bank (FSB) (t-test, $t(19) = -0.99$, $p > 0.05$), although higher values were observed in the SSB (3076.9 seedlings m^{-2}) than in the FSB (1558.6 seedlings m^{-2}) (Fig. 1). Several species showed contrasting responses to storage, with *Lemna* sp., *Heteranthera limosa,* and *Azolla* sp. presenting higher densities in SSB, while other taxa emerged exclusively in FSB (Table 1).

Experiment 2. Effect of temperature on the emergence of seedlings

Seed bank samples exposed to contrasting temperature regimes exhibited pronounced differences in species richness, taxonomic composition, and biological forms (Table 2). Germination at 37°C (SB37) resulted in the emergence of nine taxa distributed among five families, whereas only three taxa belonging to three families emerged at 17 °C (SB17). Free-floating and emergent life forms predominated under SB37 conditions, while germination at SB17 was restricted mainly to emergent and amphibious forms.

At 37°C, *Lemna* sp. and *Azolla* sp. were the most abundant taxa, with densities of 2082.69 and 746.15 seedlings m^{-2}, respectively, followed by *Heteranthera limosa* (355.77 seedlings m^{-2}). In contrast, seedling emergence at 17 °C was extremely limited, with only *Lemna* sp., *H. limosa,* and one morphospecies recorded, all presenting very low densities (≤ 7.69 seedlings m^{-2}) (Table 2).

Temperature had a significant effect on total seedling density (GLM, $p < 0.001$). The average density observed under SB37 conditions (3305.77 seedlings m^{-2}) was more than 200 times higher than that recorded at SB17 (11.53 seedlings m^{-2}) (Fig. 1). Pairwise comparisons confirmed significant differences between the two temperature treatments.

Table 1. List of species of aquatic plants, biological forms (BF), density (seedlings/ m^2) by species and richness (S) of Fresh seed bank (FSB) and stored seed bank (SSB). Biological forms (Fb): fixed submerged (SF); free submerged (SL); fixed floating (FF); free floating (FL); amphibious (A); emergent (E). Fresh seed bank samples (FSB), stored seed bank (SSB).

Family/Species	BF	Treatments	
		FSB	SSB
Amaranthaceae			
Alternanthera philoxeroides (Mart.) Griseb.	A	0.62	–
Araceae			
Lemna sp.	FL	198.77	1000.00
Pistia stratiotes L.	FL	145.06	28.85
Characeae			
Chara sp.	SF	17.28	–
Cyperaceae			
Eleocharis elegans (Kunth) Roem. & Schult.	E	0.62	–
Eleocharis minima Kunth	E	1.85	–
Nymphaeaceae			
Nymphaea sp.	FF	48.15	7.69
Onagraceae			
Ludwigia octovalvis (Jacq.) P.H. Raven	A	16.67	–
Ludwigia peploides (Kunth.) P.H. Raven	E/A	27.16	–
Ludwigia sp. 1	A	–	178.85
Poaceae			
Poaceae sp. 1	E	4.94	–
Poaceae sp. 2	E	3.09	–
Poaceae sp. 3	E	1.85	–
Pontederiaceae			
Heteranthera limosa (Sw.) Willd.	E	332.10	405.77
Salviniaceae			
Azolla sp.	FL	751.23	1430.77
Morphospecies			
Morphospecies sp. 1	E/A	9.26	–
Morphospecies sp. 2	E/A	–	25.00
Species richness (S)		15	7

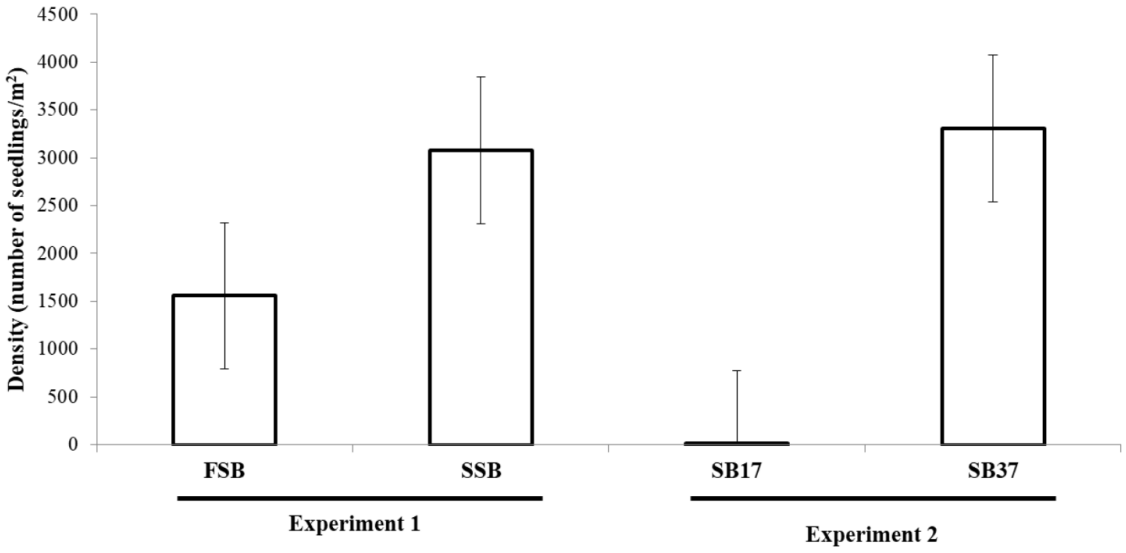


Figure 1. Total seedling density (mean ± standard error) according to treatments in Experiments 1 (Effect of seed bank storage on the emergence of seedlings) and 2 (Effect of temperature on the emergence of seedlings). FSB: fresh seed bank; SSB: stored seed bank; SB17: seed bank germinated in a growth room at a constant temperature of 17°C; SB37: seed bank germinated at an average temperature of 37°C.

Table 2. List of species of aquatic plants, biological forms (BF), density (seedlings/ m^2) by species and richness (S) of the seed bank placed to germinate at 17°C (SB17) and 37°C (SB37). Biological forms (BF): fixed submerged (SF); free submerged (SL); fixed floating (FF); free floating (FL); amphibious (A); emergent. Germination room at constant temperature of 17 °C (SB17), germination with average temperature of 37 °C (SB37).

Family/Species	BF	Treatments	
		SB17	SB37
Araceae			
Lemna sp.	FL	1.92	2082.69
Pistia stratiotes L.	FL	–	3.85
Characeae			
Chara sp.	SF	–	3.85
Onagraceae			
Ludwigia sp. 2	A	–	78.85
Ludwigia sp. 3	FF	–	11.54
Ludwigia sp. 4	A	–	3.85
Poaceae			
Poaceae sp. 4	E	–	19.23
Pontederiaceae			
Heteranthera limosa (Sw.) Willd.	E	1.92	355.77
Salviniaceae			
Azolla sp.	FL	–	746.15
Morphospecies			
Morphospecies sp. 1	E/A	7.69	–
Species richness (S)		3	9

Discussion

Effect of seed bank storage on the emergence of seedlings

Seed banks in pond sediments represent a natural strategy for preserving plant biodiversity, especially in dynamic or disturbed habitats (Wakshum *et al*., 2018). These banks act as a genetic reservoir for vegetation regeneration in subsequent hydrological cycles or in degraded areas (Wang *et al*., 2015; Carta, 2016). Our findings demonstrate that the seed bank preserved its germination capacity both after three years and after four years of cold storage, under favorable conditions. This long-term persistence highlights the potential use of sediment seed banks for the restoration of aquatic environments and for the *ex situ* conservation of native aquatic plants.

The storage conditions adopted, sediment samples collected during the natural dry phase of the pond, and sealed in plastic bags, likely helped preserve seed germinability by preventing moisture fluctuation and limiting gas exchange (Mbofung *et al*., 2013). Additionally, low temperatures reduce seeds' metabolic activity, preventing aging by oxidation and degradation of membranes (Silva *et al*., 2022b). This conservation strategy is particularly suitable for orthodox seeds, which are characterized by their ability to tolerate desiccation and remain viable under low-moisture, low-temperature conditions (Mayrinck *et al*., 2019). According to available reports, a substantial proportion of macrophyte species exhibit orthodox seed behavior (Hay *et al*., 2000), which may partially explain the potential for long-term cold storage observed in our study. The comparable emergence patterns of FSB and SSB (Table 1) suggest that seeds in this temporary seed bank are resilient to long-term storage under cold conditions, corroborating previous findings on aquatic plant species stored at low temperatures (Zhao *et al*., 2017). Interestingly, the density of *Lemna* sp. markedly increased after storage (Table 1), rising from 198.77 individuals in the fresh seed bank to 1000.00 in the stored seed bank. This pattern may indicate a possible dormancy release promoted by prolonged exposure to low temperatures, resembling cold stratification effects reported for some aquatic species. However, since dormancy mechanisms were not directly evaluated in this study, this interpretation should be considered with caution.

Despite the maintenance of germination capacity, a decline in species richness was observed after storage. It is important to emphasize that the evaluation of the seed bank was based exclusively on seedling emergence. We did not perform direct viability tests on the remaining sediment after the experiment; therefore, it was not possible

to determine whether non-emerging propagules were dead or remained dormant under the tested conditions. The absence of germination in certain species may be related to factors such as the death of vegetative propagules, as described for seeds of *Ocotea puberula* (Rich.) (Vicente *et al.*, 2016), or to the presence of secondary dormancy, as observed in species of *Beta vulgaris* L. (Hourston *et al.*, 2022). For some species, such as *Alternanthera philoxeroides*, *Ludwigia octovalvis*, *L. peploides*, *Eleocharis elegans*, and *E. minima*, the low seedling emergence recorded in the FSB suggests that these species were already present at low densities in the sediment bank. Species with initially low emergence may be more susceptible to local disappearance after prolonged storage due to reduced propagule persistence or viability loss over time. Additionally, sensitivity to cold temperatures (*Pistia stratiotes*, *Chara* sp.) and intrinsically low seed longevity (*Nymphaea* sp.) may have contributed to species loss (Kan & Song, 2008; Sederias & Colman, 2007; Dalziell *et al.*, 2019).

Ecological significance and conservation under environmental change

Beyond their ecological value for biodiversity maintenance, temporary ponds also offer essential ecosystem services, such as water provision for human use, livestock, and agriculture (Lumbreras *et al.*, 2016). However, anthropogenic pressures, such as water extraction and agriculture in nearby areas, can eutrophicate these habitats and reduce their hydroperiods, affecting plant reproductive success and seed production (Bouahim *et al.*, 2014). Climate change further intensifies these challenges, with increased temperatures and prolonged droughts leading to shorter inundation periods and greater desiccation risk (Bagella *et al.*, 2016; Grillas *et al.*, 2021). Under these scenarios, *ex situ* storage of seed banks emerges as a vital conservation strategy, enabling the preservation of viable propagules that might otherwise be lost during extreme climate events. Our findings support the feasibility of storing sediment-derived seed banks for at least three to four years, providing a reservoir for future restoration efforts in ephemeral wetlands.

Germination under elevated temperature scenarios

In the most optimistic climate change scenario for Brazil's semi-arid region, average annual air temperature is expected to increase by at least 1.5 °C, and in the worst scenario, by up to 5 °C, reaching 32 °C by 2040 (IPCC, 2021). In this context, our results (Fig. 1, Table 2) revealed that seeds remained viable and germinated at both tested temperatures (17 °C and 37 °C); however, since only two temperature points were evaluated, no inference about thermal limits (Tb or Tmax) can be drawn. This aligns with evidence that aquatic macrophytes exhibit broad thermal tolerance, occupying habitats ranging from extreme deserts (~58 °C) to subpolar zones (Murph *et al.*, 2019). It is worth noting that fewer than 2% of macrophyte species have a global distribution, indicating that thermal tolerance is often linked to local adaptation (Murph *et al.*, 2019).

Previous studies have reported that germination in aquatic macrophytes generally occurs within a temperature range of approximately 15 to 40 °C, with an optimum near 24 °C (Baskin & Baskin, 2014), although these thresholds may vary considerably among species. Our results indicate that several species present in the studied pond are likely adapted to the local tropical semi-arid climate, where daily temperatures frequently exceed 30 °C (Lucena *et al.*, 2021). This may have led to the high germination density observed under SB37 conditions (Fig. 1, Table 2). Temperature effects on seed germination reflect species-specific physiological adaptations to local environmental conditions and ecological niches (Oliveira *et al.*, 2014). In the Caatinga, for example, native dry forest species often exhibit optimal germination near 35 °C (Dantas *et al.*, 2020). The combined effects of seasonality, hydrological variation, and alternating drought/flooding cycles likely promote the selection and development of physiologically resilient seed banks in these regions.

Species-specific responses and implications for management

In all experimental conditions (FSB, SSB, SB17, and SB37), both *H. limosa* and *Lemna sp*. successfully germinated. While *H. limosa* is known to produce dormant and persistent seeds, species within the genus *Lemna* often rely on vegetative reproduction and a short life cycle (Rejmánková, 1976; Baskin *et al.*, 2003). Nonetheless, both species have global distributions and appear to exhibit broad thermal germination windows, as confirmed by their performance at 17 °C (Table 2). Among them, *Lemna* sp. showed significantly higher germination at 37 °C, which may also indicate thermotolerance or ecological plasticity (Ziegler *et al.*, 2023).

The considerable density of *Azolla* in both experiments also deserves attention. As an aquatic fern, *Azolla* reproduces through resistant sporocarps that can persist in sediments during dry phases (Dyer, 1994). Its consistent emergence in both fresh and stored seed banks suggests effective survival of these propagules under cold storage conditions. This resilience may contribute to rapid recolonization following rewetting events, reinforcing the ecological importance of sediment banks for maintaining functional diversity in temporary ponds.

Interestingly, the difference in seedling density between SSB (27 °C) and SB37 was relatively small (~229 seedlings/m^2), reinforcing the notion that seed banks from temporary ponds in semi-arid regions can retain germinability for at least four years when stored under cold conditions (Fig. 1).

Practical applications for conservation and restoration

The successful germination of seed banks after prolonged cold storage reinforces the potential use of sediment seed banks as an accessible and cost-effective source of native plant material for ecological restoration. In semi-arid regions, where hydrological variability and habitat degradation increasingly threaten aquatic biodiversity, sediment-based seed banks may serve as natural repositories of local genetic diversity (Brock, 2011; Faist *et al*., 2013; Fernández-Zamudio *et al*., 2018). These banks can be integrated into regional germplasm collections to support the conservation of aquatic macrophytes adapted to temporary water bodies. Moreover, their use in restoration initiatives, such as the rewetting of degraded wetlands or the recovery of seasonal ponds, could enhance the resilience and ecological integrity of these systems by ensuring the reestablishment of native species.

Our findings indicate that the seed bank as a functional unit retained overall germinability after prolonged cold storage and maintained its capacity for regeneration under elevated germination temperatures, provided water availability persisted for at least six weeks. Although a reduction in species richness was observed, the persistence of multiple taxa suggests that the seed bank remained ecologically viable. Cold storage thus represents a practical and scalable tool for the conservation and restoration of aquatic plant communities in semi-arid environments. Nevertheless, further research in establishing protocols for the collection, storage, and germination of sediment seed banks, while considering temperature thresholds and species-specific responses, may significantly contribute to long-term conservation planning, particularly in regions like the Brazilian semi-arid, where aquatic habitats are highly dynamic and understudied. Additionally, we recommend conducting similar studies across a larger number of temporary ponds throughout different semiarid regions to further validate our findings regarding seed banks in these environments.

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Authors' Contributions

Raquel Araujo Gomes: conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing – original draft and writing – review & editing; Jailton de Jesus Silva: formal analysis, methodology, visualization and writing – review & editing; Jamille Cardeal da Silva – methodology, visualization and writing – review & editing; Maria Carolina Tonizza Pereira: investigation, methodology, visualization, writing – original draft and writing – review & editing; Bárbara França Dantas: investigation, methodology, supervision, visualization, writing – original draft and writing – review & editing

Conflict of Interest

There are no conflicts of interest (personal, scientific, commercial, political, or financial) in the submitted manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available in the repository: Gomes, Raquel Araujo; Silva, Jailton de Jesus; Silva, Jamille Cardeal da; Pereira, Maria Carolina Tonizza; Dantas, Bárbara França, 2026, "Influence of storage and temperature on the germination of a macrophyte seed bank from a temporary pond in the Brazilian semiarid", https://doi.org/10.48331/SCIELODATA.HVCYBX, SciELO Data,VERSÃO RASCUNHO

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