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PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

# Effects of burial and storage on germination and seed reserves of 18 tree species in a tropical deciduous forest in Mexico

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**Abstract** The changes in germination and seed reserve composition that occur while seeds are stored in the laboratory or buried in the soil are important for understanding the potential and ecological longevity of seeds as well as seed-bank dynamics. Both germination and seed-bank dynamics depend on water availability. We studied 18 tree species, including those with permeable or impermeable seeds, from a tropical deciduous forest in Mexico. We measured seed germination in a growth chamber after (1) dispersal, (2) laboratory storage, (3) seed burial at two field sites and directly in the field, and (4) two rainy seasons. Lipids, nitrogen, and nonstructural carbohydrates were quantified after dispersal and after laboratory or field storage. Sixteen species were viable after three periods of laboratory storage (~3 years). Eleven species were viable after two burial periods in the field (~2 years). Nitrogen concentration decreased after storage and burial in 11 species. Species lipid concentration had a negative relationship with species water content at dispersal and after one burial period, whereas nonstructural carbohydrates showed the opposite trend. Potential and ecological longevities were similar in impermeable seeds. Most of the species studied can form persistent seed banks consisting mainly of species with impermeable seeds that can remain in the soil without degrading their viability. Germination in the field is staggered following natural precipitation pulses as a strategy to stagger seedling recruitment, which may insure against unfavorable conditions.

**Keywords** Seed bank · Ecological longevity · Potential longevity · Field germination

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## Introduction

After dispersal, seeds can germinate, remain in the soil seed bank, or die. Soil seed banks (SSBs) play a crucial role in vegetation dynamics (Van der Valk and Pederson 1989). In SSBs, the lifespan of the seeds is determined by their genetic and morphophysiological attributes, as well as by the interaction of seeds with biotic and abiotic factors (Blaney and Kotanen 2001). Some of the features that may extend seed longevity in field conditions (ecological longevity) are small seed size, the presence of chemical and physical defenses, dormancy, and persistence (Vazquez-Yanes and Orozco-Segovia 1993; Baskin and Baskin 1998; Thompson et al. 2003; Gardarin et al. 2010).

In all plant communities, there are species with seeds that take up water (are permeable) and those whose seeds have covers that impede water uptake (impermeable seeds). Due to their covers, physical dormancy is seen in impermeable seeds, and this dormancy typically leads to longer survival of these seeds in the SSB, whereas permeable seeds have a shorter lifespan in soil (Jankowska-Błaszczuk and Grubb 1997). Impermeable seeds are frequently found in tropical deciduous forest (TDF; Khurana and Singh 2001) because of the abundance of plant families in orders where physical dormancy is common, namely Fabales (Baskin and Baskin 1998).

In the SSB or during laboratory storage, deteriorative changes related to aging occur in seeds, including reduced enzyme activity, the appearance of free-radical activity, lipid peroxidation, and a decrease in stored reserves, such as N-compounds, lipids, and carbohydrates (Telewski and Zeevaart 2002; Rajjou and Debeaujon 2008). As result of this deterioration, some seeds become unable to germinate, and those that do germinate produce weak or abnormal seedlings (Villiers 1974; Witkowski and Wilson 2001; Sánchez-Coronado et al. 2007). In nature, reserve depletion can occur due to the increase in metabolism that takes place during partial seed imbibition, although this allows for seed reserve mobilization, favoring fast and uniform germination when environmental conditions are favorable (Gamboa-deBuen et al. 2006). The stored reserves, water content, and tolerance of dryness of seeds determine the length of time that seeds can remain viable in the SSB (ecological longevity) or under optimal storage conditions (potential longevity; Vazquez-Yanes and Orozco-Segovia 1993).

After dispersal and seed permanence in the SSB, seeds may germinate or remain for several years. Field germination is regulated by environmental cues such as temperature, light, and water availability, which vary in space and time. These cues indicate that adequate timing of germination and seedling establishment are key factors in community dynamics (maintenance and renewal) and composition (Vazquez-Yanes and Orozco-Segovia 1993).

In recent years, most studies of germination have been directly related to the tropical lowland rain forest, where the germination of most species is related to canopy gaps and where seedlings are strongly shade tolerant at the establishment stage (Jankowska-Błaszczuk and Grubb 1997). However, seed germination ecology in TDFs has been poorly studied, which may lead to risks when basing TDF regeneration and restoration on current knowledge of the tropical rain forest (Vieira and Scariot 2006), even though the main constraint on germination and seedling establishment in the TDF is the water supply.

Tropical deciduous forests are highly threatened ecosystems and are characterized by a marked dry season that can last from 2 to 8 months (Khurana and Singh 2001). In seasonal ecosystems, 76 % of the species produce dormant seeds (Baskin and Baskin 1998), and staggered germination during the growth season has been reported (Garwood 1983). The Chamela Biological Station is on the Pacific coast of Mexico (19°30′N, 105°03′W). The mean annual temperature in this area is 24.9 °C, and the mean annual precipitation is 788 mm (1977–2003). On average, 80 % of the annual precipitation occurs between July and October (Bullock and Solis-Magallanes 1990).

Several seed traits (seed reserves, structure, morphology, and physiology) are key influences on seed longevity and germination. While these traits have been studied separately, the relations between them have not, and the great differences seen in such research between the laboratory and field have not been discussed (Orozco-Segovia and Vázquez-Yanes 1989; Orozco-Segovia et al. 1987). Thus, we performed an integrative study of the expression of seed functional diversity in the laboratory and field from seed dispersal to seed germination. Knowledge of the changes in germination and seed reserve composition during seed burial and storage will allow us to define the relevance of the SSB to the TDF. A lack of knowledge about seed biology is one of the main challenges in attempts to preserve the TDF. We studied 18 tree species, and we asked the following questions. (1) In the species studied, is the potential longevity different from the ecological longevity? (2) Are the ecological and potential longevities of impermeable seeds different from those of permeable seeds? (3) In impermeable and permeable seeds after storage or burial, does germination differ from that seen at dispersal? (4) In impermeable and permeable seeds after storage or burial, is the reserve composition different from that at dispersal? (5) In the field, do rain pulses determine germination?

## Materials and methods

### Study site and seed collection

In 2006, mature seeds from at least 10 different individuals of each of 18 tree species (Table 1) were collected at the peak of the dispersal season at the Chamela Biological Station (19°30′N, 105°03′W) on the Pacific coast of Mexico. In the Chamela TDF, seed dispersal occurs across the dry season; the seeds of some species may remain in the soil for 10 months, whereas the seeds of other species are dispersed a few weeks before the rainy season (Bullock 1986). Dispersal time and the presence of dormancy in several species suggest the presence of a SSB (transient or permanent) in the TDF of Chamela (Maza-Villalobos et al. 2011). In the soil, seeds are exposed to dew produced by the condensation of evapotranspiration occurring prior to the rainy season (Barradas and Gonzalez-Medellín 1999) and to the "canicula" (winter rains); both can increase soil moisture availability and may favor partial or total seed imbibition before the rainy season. In addition, dry spells are common during the rainy season (Páramo-Pérez 2009) in the TDF, which delays seed germination (Engelbrecht et al. 2006).

### Seed dry mass and water content

The dry-basis water content (WC) was determined at the time of dispersal based on the fresh seed and dry mass data reported by Soriano et al. (2011). The WC was also determined after one period (from seed dispersal to the

**Table 1** List of studied species, families of those species, peak dispersal times (collection dates were in 2005 and 2006), times from seed dispersal to the first rainy season, and seed permeabilities (impermeable or permeable)

| | Species | Family | Collection date | Time (days) | Permeability |
|---|---|---|---|---|---|
| 1 | *Acacia farnesiana* | Fabaceae | March | 110 | I |
| 2 | *Acacia sp.* | Fabaceae | March | 110 | I |
| 3 | *Apoplanesia paniculata* | Fabaceae | Nov[a] | 230 | P |
| 4 | *Caesalpinia coriaria* | Fabaceae | Jan | 170 | I |
| 5 | *Caesalpinia eriostachys* | Fabaceae | April | 35 | P |
| 6 | *Caesalpinia platyloba* | Fabaceae | Jan | 170 | I |
| 7 | *Ceiba pentandra* | Bombacaceae | May | 27 | P |
| 8 | *Coccoloba barbadensis* | Polygonaceae | March | 110 | P |
| 9 | *Cochlospermum vitifolium* | Cochlospermaceae | May | 27 | I |
| 10 | *Enterolobium cyclocarpum* | Fabaceae | May | 27 | I |
| 11 | *Gyrocarpus jatrophifolius* | Hernandiaceae | April | 35 | P |
| 12 | *Hintonia latiflora* | Rubiaceae | March | 110 | P |
| 13 | *Ipomoea wolcottiana* | Convolvulaceae | April | 27 | I |
| 14 | *Lonchocarpus eriocarinalis* | Fabaceae | Jan | 170 | P |
| 15 | *Pithecellobium dulce* | Fabaceae | March | 110 | P |
| 16 | *Ruprechtia fusca* | Polygonaceae | Nov[a] | 230 | P |
| 17 | *Swietenia humilis* | Meliaceae | May | 27 | P |
| 18 | *Tabebuia rosea* | Bignoniaceae | May | 27 | P |

[a] Collection date was in 2005; otherwise, collection date was in 2006

following rainy season for each species) of laboratory storage or seed burial.

### Seed storage and burial

To evaluate the effects of time and microsite on germination and seed reserve composition, we stored the seeds of each species at three sites. One batch of at least 200 seeds was stored in closed plastic bottles under semi-controlled conditions (i.e., in a laboratory) in the darkness. The ambient average temperature was 16–24 °C, and the relative humidity (RH) was 35–50 %. Each species was stored in the laboratory from the date of dispersal to the beginning of the first and third rainy seasons in the TDF, designated 1LP and 3LP, for approximately 1 and 3 years, respectively. Additionally, two batches of at least 400 seeds were buried: one in an open site (2 $m^2$ in size) covered with annuals and grasses but deprived of trees, and the other in a closed site with a continuous canopy. In each site, each lot (~100 seeds) was enclosed in a nylon mesh bag at a depth of 3 cm. Seeds of each species remained buried from their dispersal to the beginning of the first or the second rainy season, designated one burial period (1BP) and two burial periods (2BP), respectively.

### Microclimate

During the first dry season in the two field sites, we recorded three parameters: the soil moisture at depths of 5 and 15 cm with a PR1/4 profile probe (Delta-T Devices Ltd., Cambridge, UK); the temperature and RH at the soil surface, using three HOBO U23-001 data loggers per site (Onset Computer Corporation, Pocasset, MA, USA); and the soil temperature (at a depth of 3 cm) using three HOBO H01-001-01 data loggers per site (Onset Computer Corporation). For each site, we obtained the global site factor (GSF) using hemispheric photographs (Valladares 2006). The photos were taken at the soil surface using a camera (CoolPix 995, Nikon, Tokyo, Japan) with a fish-eye lens. The images were processed using the Hemiview v.2.1 software package (Delta-T Devices Ltd.). The GSF was calculated from values of direct and diffuse radiation ranging from 0 to 100 %. We obtained precipitation data from the meteorological station of Chamela. Temperature sensors were lost during the first rain due to superficial water runoff; the soil temperatures and RH in the rainy season were calculated from air temperatures, and soil moisture was calculated using a regression analysis (see the Electronic supplementary material, ESM).

### Germination in growth chambers

To determine the germination capacities and lag times of the 18 species studied, germination tests were conducted after dispersal. Three replicates were performed per species, and 30 seeds per replicate were used. The seeds were germinated in a growth chamber (Lab-Line, Lab-Line Instruments, Inc., Melrose Park, IL, USA) at 25–30 °C in 12/12 h of light/dark. Prior to all germination treatments, we tested whether the seeds took up water by immersing them in water for 48 h and placing the seeds on agar plates for 6 months. Seven of the species tested had an

impermeable seed coat (*Acacia farnesiana*, *Acacia* sp., *Caesalpinia coriaria*, *C. eriostachys*, *C. platyloba*, *Enterolobium cyclocarpum*, and *Ipomoea wolcottiana*) and were considered physically dormant. The seeds of these species were scarified with 98 % $H_2SO_4$ to test their germination capacities in growth chamber experiments. After 1LP and 3LP, we conducted seed germination tests as described above. After 1BP and 2BP, at the beginning of the rainy season, a bag of seeds of each species was exhumed. Three replicates of 30 seeds per replicate were air-dried in the shade for 2 days and germinated in the growth chambers.

### Germination in the forest soil

To test germination in the TDF soil, after 1BP and 2BP, the unearthed seeds of each species were placed in their respective site (open or closed) inside nylon mesh boxes (11 × 11 × 5 cm), with three replicates of 30 seeds per replicate studied. The boxes were filled with 3 cm of soil and buried; the boxes were then covered with removable nylon mesh tape and covered with soil or litter, depending on the local soil-surface conditions. During the first rainy season (1RS), seed germination was recorded every 3 days. Germination was characterized by radicle protrusion. In the second rainy season (2RS), field germination was recorded each week. Seed availability limited the number of experiments.

### Quantification of seed reserves

We measured the seed reserves (lipids, N, and nonstructural carbohydrates) at dispersal (initial data are available in Soriano et al. 2011), with three replicates employed per analysis and per species. The reserve concentration was determined from the percentage embryo and extra-embryonic tissue. After 1BP and 1LP in the open and closed sites, the seeds were dried in an oven at 55 °C, the embryo and extra-embryonic tissues were separated, and the reserves were quantified according to techniques described by Soriano et al. (2011).

### Statistical analyses

Cumulative germination curves were fitted using the Table Curve 2D v.3 program (AISN Software, Chicago, IL, USA). The lag time was obtained from the fitted curves. The differences in germination percentages, lag times, and seed reserve compositions among the treatments were tested with a two-way ANOVA test, using species and storage site as factors. The Tukey test was used to make post hoc comparisons. Statistical analyses were conducted using the software package Sigma Plot v.11 (Systat Software, Richmond, CA, USA). Regression analyses between reserve and seed WC were conducted using Table Curve 2D v.3. Germination percentages were arcsine transformed to satisfy the assumptions of the tests.

## Results

### Microclimate

Precipitation during this 2-year study showed an erratic pattern that characterizes this region. At depths of 5 and 15 cm, soil moisture in the closed site was higher than that in the open site at midday during the first rainy season. Throughout the year, the GSF and temperature fluctuations (at the soil surface and at a depth of 3 cm) were higher in the open site than in the closed site (Fig. 1). The soil surface reached an RH of 100 % for up to 8 h overnight in both sites during 1BP (Table 2).

### Germination of impermeable seeds in the growth chambers

At the time of dispersal, seeds from the seven species with impermeable seed coats did not germinate without scarification. Once scarified, from 23 to 100 % (*Cochlospermum vitifolium* and *Acacia* sp., respectively) of the seeds of these species germinated. After 1LP, species with impermeable seeds maintained high germination percentages after scarification (60–100 %). Additionally, there were significant increases in the germination percentages of *Caesalpinia platyloba* and *C. vitifolium*, whereas those of *E. cyclocarpum* and *C. coriaria* seeds decreased significantly but remained above 50 % ($F_{6, 24} = 44$, $P < 0.05$; Fig. 2).

Impermeable seeds germinated without scarification after 1LP. Their germination percentage was lower than that of the scarified seeds, except in *C. coriaria*. For *A. farnesiana*, *Acacia* sp., *C. platyloba*, *C. vitifolium*, and *I. wolcottiana*, germination was lower than 10 %, whereas for *C. coriaria* and *E. cyclocarpum*, germination was between 41 and 55 %. After 3LP, the seven species with impermeable seed coats achieved >89 % germination once scarified. The non-scarified seeds of four species—*C. coriaria*, *C. platyloba*, *C. vitifolium*, and *I. wolcottiana*—germinated, whereas those of *A. farnesiana*, *Acacia* sp., and *E. cyclocarpum* did not (Fig. 2).

After the impermeable seeds were buried (1BP and 2BP), the germination percentage after scarification was high (>50 %). After 1BP, the non-scarified seeds of the seven species showed different germination percentages. *A. farnesiana*, *Acacia* sp., *C. vitifolium*, and *I. wolcottiana* presented low germination percentages (5–17 %) and did not differ between the treatments (Fig. 3; $F_{5, 55} = 17.2$, $P < 0.05$), whereas *C. coriaria*, *C. platyloba*, and *E. cyclocarpum* exhibited 35, 78, and 57 % germination,

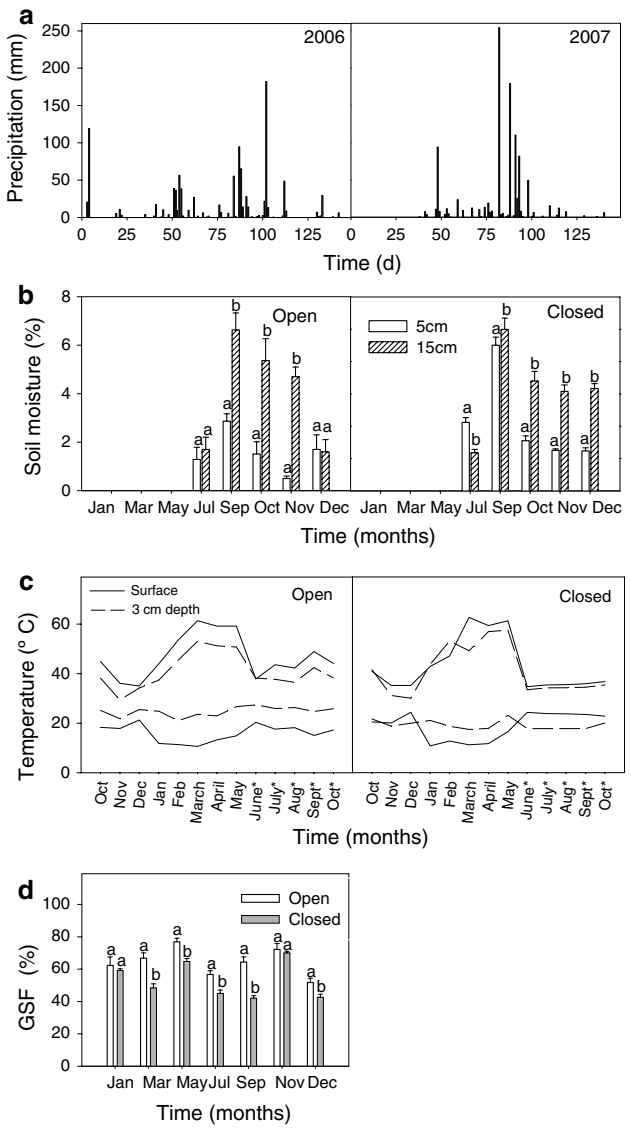


**Fig. 1** **a** The amount of precipitation measured from June to October in 2006 and 2007. **b** Soil moisture (mean ± SE) at depths of 5 and 15 cm in the open site and the closed site. **c** Soil temperature (°C) at the soil surface and at a depth of 3 cm in the open and closed sites. **d** Global site factor (*GSF*; mean ± SE) in the open and closed sites. *Letters* denote significant differences between pairs (*t* test, $P < 0.05$)

respectively. After burial, only *E. cyclocarpum* showed a higher germination percentage and faster germination in the open site than in the closed site. After 2BP, *C. coriaria* seeds were predated, but the six remaining species germinated without scarification (5–25 %; Fig. 3).

## Germination of permeable seeds in the growth chambers

At the time of dispersal, the seeds of permeable species germinated without any treatment (from 33 % in *Apoplanesia paniculata* to 100 % in *C. eriostachys*; Fig. 4). After

**Table 2** Time (hours per day) that the air at the soil surface achieved 100 % relative humidity in the dry season for the two field sites

| | Open | Closed |
|---|---|---|
| October | 8 | 8 |
| November | 7 | 8 |
| December | 8 | 8 |
| January | 8 | 8 |
| February | 6 | 7 |
| March | 3 | 4 |
| April | 3 | 3 |
| May | 3 | 2 |

1LP, *A. paniculata*, *Ceiba pentandra, Pithecellobium dulce, R. fusca*, and *Swietenia humilis* had decreased germination percentages; *Hintonia latiflora* had an increased percentage; and *C. eriostachys*, *Coccoloba barbadensis*, *Gyrocarpus jatrophifolius*, *Lonchocarpus eriocarinalis*, and *Tabebuia rosea* did not exhibit any change in their germination percentages. After 3LP, only nine species germinated: *S. humilis* and *C. barbadensis* did not germinate. *A. paniculata* and *R. fusca* presented increased germination percentages compared with those seen at the time of dispersal and after 1LP. *C. pentandra*, *G. jatrophifolius*, *L. eriocarinalis*, *P. dulce*, and *T. rosea* exhibited decreased germination percentages, whereas the germination percentages of *C. eriostachys* and *H. latiflora* did not differ from those seen in the previous test ($F_{10, 20} = 10.6$, $P < 0.05$; Fig. 4).

After 1BP, all of the species with permeable seeds germinated in growth chambers (Fig. 5). The germination percentage of *A. paniculata* increased with respect to the germination achieved at the time of dispersal (Fig. 4). In contrast, *C. eriostachys*, *C. pentandra*, and *G. jatrophifolius* presented decreased germination percentages, and *C. barbadensis*, *H. latiflora*, *L. eriocarinalis*, *P. dulce, R. fusca*, *S. humilis*, and *T. rosea* did not show any change in their germination percentages ($P < 0.05$).

After 2BP, only five species germinated. In comparison with germination after 1BP, the germination percentages of *C. pentandra* (in the closed site) and *G. jatrophifolius* (in the open site) increased, the germination percentages of *C. barbadensis* and *R. fusca* (both in the open site) decreased, and the germination percentage of *A. paniculata* did not change ($P < 0.05$).

## Germination of impermeable seeds in the field

Seeds were not scarified for these experiments. In the field, during the 1RS, the seeds of *C. platyloba* and *E. cyclocarpum* germinated in low percentages (2–6 %), while the *Acacia* sp. (17 %) and *C. coriaria* (25 %) seeds germinated only in the open site. The other species did not germinate in

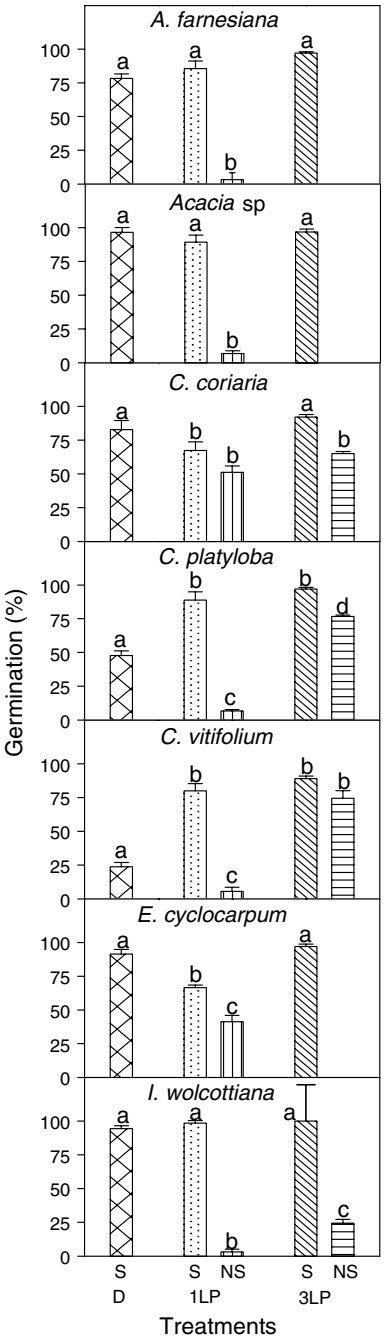


**Fig. 2** Final germination percentages (mean ± SE) of the species with scarified (*S*) and non-scarified (*NS*) impermeable seed coats at the time of dispersal (*D*) and after one or three laboratory periods (*1LP*, *3LP*). Multiple comparisons (Tukey test; $P < 0.05$) are shown for each species

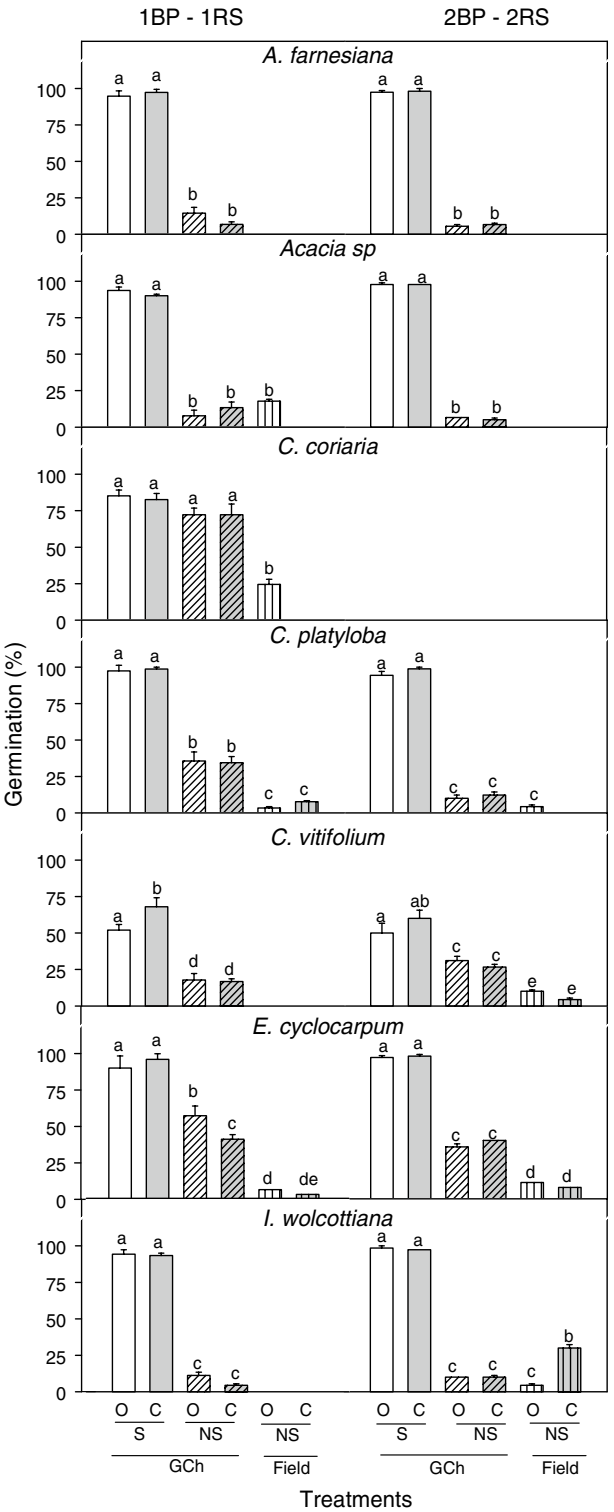


**Fig. 3** Final germination percentages (mean ± SE) of scarified (*S*) and non-scarified (*NS*) impermeable seeds in growth chambers (*GCh*) after one or two burial periods (*1BP*, *2BP*) in open (*O*) and closed (*C*) sites. Germination percentages in the first and second rainy seasons (*1RS* and *2RS*) in field conditions in open (*O*) and closed (*C*) sites are also shown. Multiple comparisons (Tukey test; $P < 0.05$) are shown for each species

the field. However, during the 2RS, the seeds of *C. vitifolium*, *E. cyclocarpum*, *I. wolcottiana*, and *C. platyloba* germinated in the closed site. Among these, during the second rainy season, only *I. wolcottiana* presented a relatively high germination percentage in the closed site (30 %), which was significantly different from its germination percentage

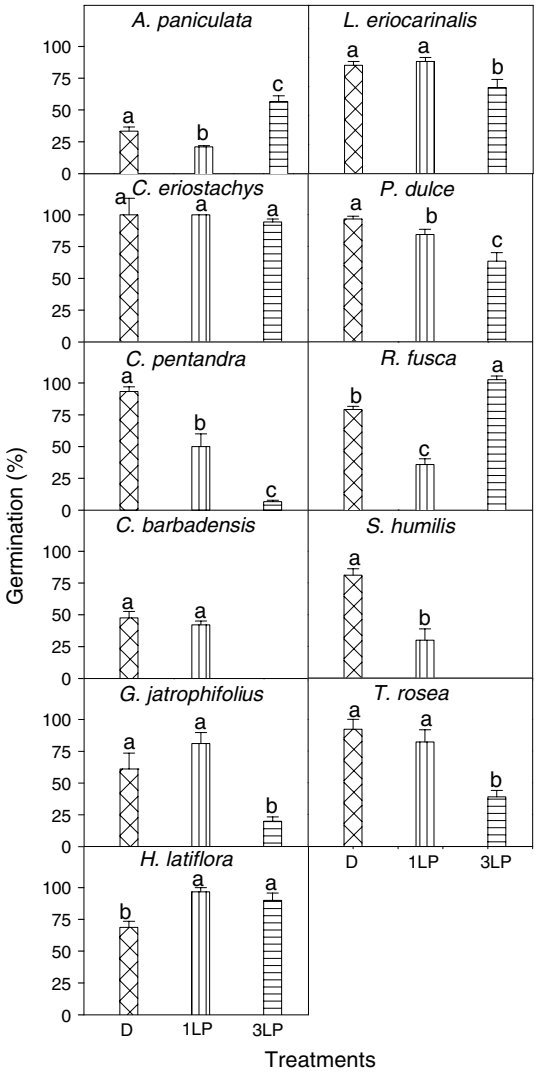


**Fig. 4** Final germination percentages (mean ± SE) of species with permeable seed coats at the time of dispersal (*D*) and after one and three laboratory periods (*1LP*, *3LP*). Multiple comparisons (Tukey test; $P < 0.05$) are shown for each species

in the open site (10 %) ($F_{5, 55} = 17.2$, $P < 0.05$; Fig. 3). In the rainy season, the field germination percentages were lower (3.3–20 %) than those reached in the growth chambers (10–78 %), except for *I. wolcottiana* in the closed site.

Germination of permeable seeds in the field

During the 1RS, the seeds of *A. paniculata*, *C. eriostachys*, *C. pentandra*, *G. jatrophifolius*, *H. latiflora*, *L. eriocarinalis, R. fusca*, *S. humilis*, and *T. rosea* germinated in both field sites, whereas the *C. barbadensis* seeds germinated only in the closed site. During 2RS, the seeds of *A. paniculata*, *C. pentandra*, *G. jatrophifolius*, and *R. fusca* germinated in the open site while *A. paniculata* only germinated in the closed site. In nine of the 11 species with permeable seeds, germination in the field was lower and started later than in the laboratory (Fig. 5; $P < 0.05$). *A. paniculata*, *C. eriostachys*, *G. jatrophifolius*, *R. fusca*, and *S. humilis* had staggered or delayed germinations (see the ESM).

Reserves

After 1LP or 1BP, in 14 of the 18 species studied, there was a decrease in the N concentration from the values found at the time of dispersal ($F_{17, 51} = 26.4$, $P < 0.05$; Table 3). *R. fusca* showed the greatest difference. After 1LP or 1BP, in *C. barbadensis, E. cyclocarpum, H. latiflora*, *L. eriocarinalis*, *P. dulce, R. fusca*, and *S. humilis*, the seed lipid concentration differed from that at the time of dispersal ($F_{17, 51} = 5.2$, $P < 0.05$; Table 3). In seven species, the lipid reserves decreased under laboratory conditions. Species with permeable seeds did not differ from those with impermeable seeds in the changes in lipids and nitrogen observed after any type of storage. The concentration of nonstructural carbohydrates (NSC) did not differ with dispersal time after 1LP and 1BP in any of the species studied. ANOVA tables and comparisons of within-species factors ($P < 0.05$) for Figs. 2, 3, 4, 5, and Table 3 are available from the author upon request. There was a negative relationship between the WC and lipid reserves in permeable seeds. A positive relationship was found between the NSC and WC; such a relationship was also found after 1BP in the open site (Fig. 6).

## Discussion

From a functional point of view, there is scant documentation of soil seed banks for the TDF (Maza-Villalobos et al. 2011), except for the Garwood studies (1989). Most studies indicate that the seed bank is scant because seeds germinate in the next rainy season or are predated (Khurana and Singh 2001). We found that in the laboratory and field, the seeds from several species remained viable for 1–3 years. Impermeable seeds maintained ~100 % germination. Thus, these species might form at least a transient (<1 year viability in the soil) or a persistent (>1 year) seed bank (Fenner and Thompson 2006).

Germination of impermeable seeds in the growth chambers

The potential longevity of the impermeable seeds (under suboptimal conditions) suggests that this did not limit the formation of an SSB in the TDF. Impermeable seeds remained viable after 1LP and 3LP (755–955 days). The increased germination percentages of scarified seeds of *C. vitifolium* and *C. platyloba* after 1LP suggests that these seeds underwent combined physiological and physical

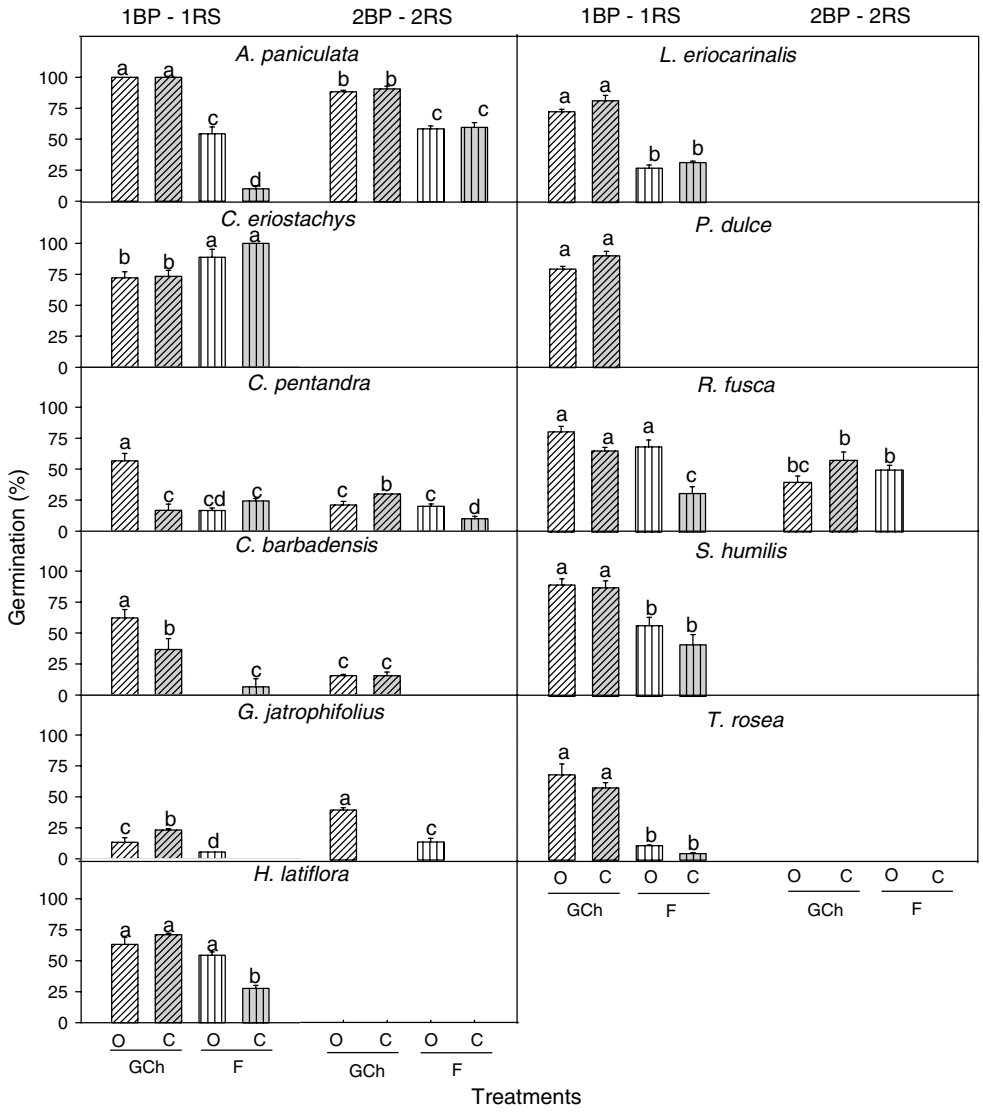


**Fig. 5** Final germination percentages of permeable seeds (mean ± SE) in growth chambers (*GCh*) after one or two burial periods (*1BP*, *2BP*) in open (*O*) and closed (*C*) sites. Germination percentages in the first and second rainy seasons (*1RS* and *2RS*) in field conditions in an open (*O*) and a closed (*C*) site are also shown. Multiple comparisons (Tukey test; $P < 0.05$) are shown for each species

dormancy (sensu Baskin and Baskin 2004), which has not previously been reported for TDF species.

Impermeable seeds are orthodox, and are thus characterized by low seed WCs and long life spans (Royal Botanical Garden Kew 2008). Both traits form a continuum between orthodox and recalcitrant seeds that varies widely under suboptimal storage conditions, such as in the laboratory. Impermeable seeds with the highest WCs (5–15 %) that germinated after 1 LP (*A. farnesiana*, *Acacia* sp., *I. wolcottiana*, *C. platyloba*, and *C. vitifolium*) lost physical dormancy faster than seeds with the lowest WCs (*C. coriaria* and *E. cyclocarpum*). After 3LP, only four species germinated without scarification (Fig. 2). This result demonstrated that seeds might have re-instated physical dormancy in the laboratory, as seen in *Ipomoea lacunose* (Jayasuriya et al. 2008). The remaining species did not germinate, but according to Baskin and Baskin (1998) and our observations, the seeds remained viable, firm, and healthy after imbibition.

After 1BP and 2BP, physical dormancy was broken to differing degrees in exhumed and non-scarified seeds. During burial, temperature fluctuations were able to dislodge the seed water gap (lens or strophiole), allowing for water uptake (Baskin 2003). Cyclic changes in physical dormancy (sensu Jayasuriya et al. 2008) might explain why some species germinated after 1BP but not after 2BP, and vice versa. Similar results were observed with seeds in laboratory storage. Variations in soil moisture and in

**Table 3** Percentage changes in seed N and lipid concentrations after one burial period (1BP) and one laboratory period (1LP)

| Species | Nitrogen | | | Lipids | | |
|---|---|---|---|---|---|---|
| | Storage site | | | Storage site | | |
| | 1BP | | 1LP | 1BP | | 1LP |
| | Open | Closed | | Open | Closed | |
| *Acacia farnesiana* | 3.0 | 5.3 | 4.2 | 8.1 | −4.1 | −11.6 |
| *Acacia sp.* | −10.7* | −10.9* | −13.1* | −9.6 | 3.7 | −5.9 |
| *Apoplanesia paniculata* | −5.2* | −8.9* | −12.4* | 4.0 | −6.2 | −0.5 |
| *Caesalpinia coriaria* | −13.6* | −12.2* | −12.3* | −9.9 | 1.0 | −0.9 |
| *Caesalpinia eriostachys* | −7.7* | −7.4* | −1.3 | 1.8 | 2.0 | −1.1 |
| *Caesalpinia platyloba* | −17.0* | −19.4* | −12.2* | 7.3 | −1.0 | −10.5 |
| *Ceiba pentandra* | −7.3* | −7.6* | −6.0* | −1.3 | −0.5 | −1.1 |
| *Coccoloba barbadensis* | −10.0 | −17.6* | −10.3 | −34.0* | −27.5* | −62.4* |
| *Cochlospermum vitifolium* | −10.8* | −7.0* | −7.2* | 5.9 | 2.6 | 6.5 |
| *Enterolobium cyclocarpum* | −12.4* | −13.5* | −11.6* | −3.9 | 3.5 | −53.7* |
| *Gyrocarpus jatrophifolius* | −8.1* | −10.6* | −12.4* | −8.9 | 0.01 | −9.3 |
| *Hintonia latiflora* | −0.6 | −0.7 | 7.9 | 7.4 | 18.7 | −32.9* |
| *Ipomoea wolcottiana* | −15.5* | −11.9* | −15.7* | −0.4 | −2.7 | −4.8 |
| *Lonchocarpus eriocarinalis* | 2.8 | 2.9 | 5.3 | −3.7 | −14.4* | 10.6 |
| *Pithecellobium dulce* | −10.5* | −2.4 | −7.7* | −16.6* | −5.2 | −13.5* |
| *Ruprechtia fusca* | −48.2* | −47.5* | −45.4* | 27.9 | 12.3 | −30.2* |
| *Swietenia humilis* | −0.7 | −0.6 | −1.9 | 1.7 | −1.5 | −4.8* |
| *Tabebuia rosea* | −6.4* | −4.1 | −9.7* | −7.0 | −5.1 | 0.7 |

The ANOVA and Tukey tests were used

* Significant differences ($P < 0.05$) compared with the concentration at the time of dispersal

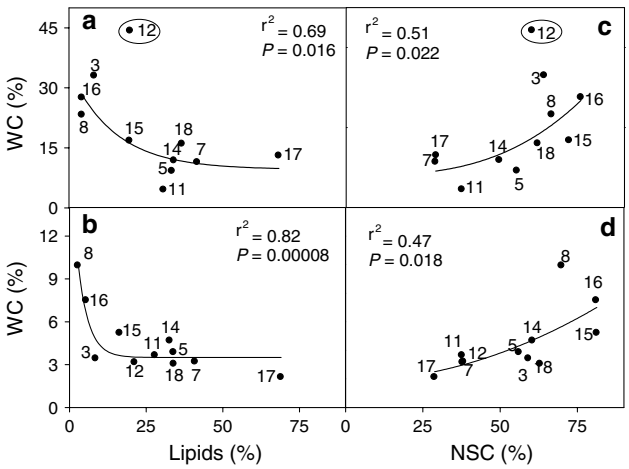


**Fig. 6a–d** Relationship between seed lipids and water content (WC) at **a** the time of dispersal ($y = 9.6 + 25.9 \exp(-x/14.7)$) and **b** after one burial period in the open site (*1BP-Open*; $y = 3.5 + 14.8 \exp(-x/3.5)$). Relationship between NSC and WC at **c** the time of dispersal ($y = 8.1 + 4.2 \exp(-0.5\ x^3)$) and **d** after one burial period in the open site (*1BP-Open*, $y = 1.9 + 0.0007 \times 2$). *Numbers* are shown according to Table 1

the laboratory during the year may explain these changes (Russi et al. 1992). Full germination of scarified seeds after burial showed that the ecological longevity might be close to the potential longevity in impermeable seeds (sensu Vazquez-Yanes and Orozco-Segovia 1993).

## Germination of permeable seeds in the growth chambers

At the time of dispersal, 11 species with permeable seeds germinated without any pre-treatment with percentages of >33 %. Permeable seeds can be morphologically or physiologically dormant or quiescent. At the time of dispersal, parts of the seed populations of *H. latifolia*, *R. fusca*, and *A. paniculata* showed physiological dormancy. After 3LP, physiological dormancy was overcome for the first two species, whereas dormancy remained in a portion of the seed population in the latter species. In *A. paniculata,* a portion of the seeds acquired secondary dormancy: the seeds did not germinate, but the imbibed seeds remained firm and did not rot, suggesting that these seeds remained viable (Baskin and Baskin 1998). In contrast, during the 3LP, the remaining seven species progressively lost their viability, similarly to the decrease in germination observed in 88 of the 100 tree species growing in the TDF in Panama after 3 years (Sautu et al. 2006).

After burial, the seeds had similar patterns to those observed after laboratory storage, but the species present in each group and the number of species differed (Fig. 3). Seeds of *Coccoloba barbadensis*, *H. latiflora*, *L. eriocarinalis*, *P. dulce*, *R. fusca*, *S. humilis*, and *T. rosea* remained viable until the beginning of the rainy season after 1BP in TDF soil. Alternatively, portions of the seed populations of *C. eriostachys*, *C. pentandra*, and *G. jatrophifolius* showed

secondary dormancy, with *G. jatrophifolius* overcoming it after 2BP. Interestingly, after 1BP, the seeds of *A. paniculata* overcame physiological dormancy, which was not attained after laboratory storage. After 2BP, *C. eriostachys*, *H. latiflora*, *L. eriocarinalis, P. dulce, S. humilis*, and *T. rosea* seeds were lost to predation or rotting, which are causes of SSB shortages in the TDF (Khurana and Singh 2001). The high abundance of *C. eriostachys* in the TDF of Chamela (Huante and Rincon 1998) may be related to the long potential longevity of its seeds (≥3LP). Based on these results, some proportions of the seeds of *A. paniculata*, *C. pentandra*, *C. barbadensis*, *G. jatrophifolius*, and *R. fusca* may form a 1–2-year SSB. Changes in germination and dormancy after burial might be related to the functional response (i.e., secondary dormancy) of seeds in a changing environment (Vleeshouwers et al. 1995). In the permeable seeds studied herein, ecological longevity was not a constraint on the creation of a temporary or persistent seed bank. However, the extent and percentage of seed viability in the soil may be shorter than in impermeable seeds.

### Germination of impermeable seeds in the field

Owing to the functional heterogeneity of the SSB, maintenance of high seed viability does not mean that a high percentage of seeds will germinate in the field. During 1RS, seeds of seven species sown close to the soil surface germinated in the field. After 2RS, the six remaining species also germinated. As observed in the laboratory, *C. platyloba* and *E. cyclocarpum* (Fig. 3) showed a loss and a re-instatement of physical dormancy, respectively. The other five species showed delayed or staggered germination (see the ESM), suggesting a gradual loss of physical dormancy due to the action of soil microorganisms on the seed cover (Morpeth and Hall 2000; Sánchez-Coronado et al. 2011).

### Germination of permeable seeds in the field

Results suggest that permeable seed loss from the seed bank is not mainly from germination, except in *C. eriostachys*, which was 100 % germinated after the 1RS in a closed site. This result showed that the safe site quality is relevant to both seed retention in the SSB and germination. The onset of germination in the field was delayed and was lower than in the laboratory (see the ESM). The fact that ~30 days separated the first rain from the establishment of the rainy season may limit the onset of germination in the TDF, as in Mediterranean ecosystems (Thanos et al. 1995). This 2-year study showed precipitation (1,059.42 and 1,032.12 mm) above the annual mean (788 mm). However, 5–14-day dry spells were observed in both years. Seeds can absorb water from a thin film of water or from water vapor coming from deeper layers of the soil (Wuest 2007); during periods without precipitation, imbibition and germination would be slower than during periods of high water availability (Garwood 1989). Staggered germination leads to staggered seedling recruitment, which might insure against eventual unfavorable conditions (Donohue 2005), such as those in Chamela (Bullock and Solis-Magallanes 1990). Dry spells during the years of this study were fewer and shorter than those in past years in which precipitation was also lower (Páramo-Pérez 2009), so the germination and establishment of the species studied may endure even harsher conditions in the TDF.

In *C. eriostachys* and *S. humilis*, the lag time between the field and laboratory did not differ. *Swietenia macrophylla* stores water in the seed coat, making it available to the embryo regardless of future rainfall (Sousa-Paiva et al. 2006). In *C. eriostachys*, a remnant of the aleurone layer might play a similar role in retaining water (D. Soriano, pers. obs). Of the five species available during the 2RS, only four germinated in the field (*G. jatrophifolius* and *R. fusca* only germinated in the open site) with a shorter lag time than in the 1RS (see the ESM). This result could be related to the advance that occurs in the germination process during burial, which results in fast germination when precipitation takes place (natural priming; Gamboa-deBuen et al. 2006).

### Reserves

In the field, after the 1BP, the seeds underwent changes in their N and lipid concentrations. The N concentration decreased in 14 of the 18 species studied. This decrease in N concentration may be related to field lixiviation, metabolic activity, and the action of reactive oxygen species, or to other aging processes (Ross 1984). The impermeable seeds also decreased N reserves, and the latter two causes might be important factors for these species. In *C. barbadensis*, the decrease in N concentration may be related to seed vigor and viability loss (Hara and Toriyama 1998). Lipid peroxidation has been reported during accelerated seed aging, but not changes in the lipid concentration (Hoshizaki and Miguchi 2005). In our work, seven species had decreased lipid concentrations after 1BP or 1LP. These species had WC >11 %, suggesting that the lipid loss might be related to metabolic activity, as shown in other species with WCs above 10 % (Pritchard and Dickie 2003). In *S. humilis* and *C. pentandra*, the loss of viability could be related to a high lipid concentration (>40 %), since lipidic seeds may be peroxidized (Bailly 2004), as previously reported for *Ceiba* species (Orwa et al. 2009).

Even if the degradation of macromolecules during seed aging is related to a reduction in germination percentages (Galleschi et al. 2002; Pukacka and Ratajczak 2007), there was no direct relationship between the decrease in N concentration and the decline in germination. However, we

do not know the extent to which the reserve depletion may have caused the loss of seed viability, or which of the species did not germinate due to secondary dormancy rather than loss of viability. Additionally, deterioration rates vary among species based on their genetic information and storage conditions (Priestley 1986; Walters et al. 2005).

In permeable seeds, at the time of dispersal, there was a negative relationship between lipids and WC and a positive relationship between NSC and WC. The fact that these relationships remained after 1BP even though some species lost more lipid reserves than others emphasizes the importance of this reserve during the early germination phases (Bewley and Black 1994). Similarly, NSC may help to maintain seed WC and retain water in TDF soil. Non-structural carbohydrates have an affinity for water in seed storage tissues, whereas lipids have less affinity for water (Pritchard and Dickie 2003).

## Conclusions

Our study shows that, for the species studied, a fraction of the seeds could survive in field conditions for at least 2 years, with some maintaining high germination percentages. Therefore, the scarcity of reported seed banks in TDFs may be related to secondary dispersal owing to the slope of the terrain and to post-dispersal predation, more than to the seed lifespan alone. The lack of a difference in germination percentage between burial sites would reflect the rank of environmental conditions where seeds can survive. Germination in the field was delayed or staggered compared with germination in growth chambers; this may increase seedling recruitment in the field, and may be used to improve restoration strategies based on a stepped reintroduction of seedlings. Lipid concentration had a negative relationship with the WC at dispersal, whereas the NSC content had a positive relationship with WC. The lipids, NSC, and water content percentages could be used to infer the potential lifespans of seeds. This approach needs to be explored for species of all ecosystems.

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