Using semi-natural and simulated habitats for seed germination ecology

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Abstract

1. Ecologically meaningful seed germination experiments are constrained by access to seeds and relevant environments for testing at the same time. This is particularly the case when research is carried out far from the native area of the studied species. 2. Here, we demonstrate an alternative - the use of glass houses in botanic gardens as simulated-natural habitats to extend the ecological interpretation of germination studies. Our focal taxa were banana crop wild relatives (Musa acuminata subsp. burmannica, M. acuminata subsp. siamea and M. balbisiana), native to tropical and subtropical Southeast Asia. Tests were carried out in Belgium, where we performed germination tests in relation to exposure to sun and foliage-shading, seed burial-depth in different heated glass house compartments, as well as seed survival and dormancy release in the soil. We anchored the interpretation of these studies by also conducting an experiment in a semi-natural habitat in the species native range (M. balbisiana - Los Baños, the Philippines), where we tested germination responses to exposure to the sun and shade. Using temperature data loggers, we determined temperature dynamics suitable for germination in both these settings. 3. In semi-natural and simulated-natural habitats, seeds germinated in response to exposure to direct solar radiation. Seed burial-depth had a significant but marginal effect by comparison, even when seeds were buried to 7cm in the soil. Temperatures at sun-exposed compared to shaded environments differed by only a few degrees Celsius. Maximum temperature of the period prior to germination was the most significant contributor to germination responses and germination increased linearly above a threshold of 23°C to the maximum temperature in the soil (in simulated natural habitats) of 35°C. 4. Glass houses can provide useful environments to aid interpretation of seed germination responses to environmental niches.

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Abstract

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Introduction

Ideally, seed germination ecology studies are carried out in both natural habitats (NHs) and laboratory conditions (LCs) (Baskin & Baskin 2014). This allows variables affecting germination to be clearly identified and ecologically interpreted. Interpretation is usually made in relation to spatial and temporal niches in NHs, or perhaps semi-natural habitats (Semi-NHs) (Table 1).

Table 1. Descriptions of environments for seed germination studies.

Name	Definition	Germination example	Control level	Interpretation level
Natural habitat (NH)	Areas composed of viable assemblages of plant and/or animal species of largely native origin and/or where human activity had not essentially modified an area's primary ecological functions and species composition. [a]	(Dinsdale, Dale & Kent 2000)		
Semi-natural habitat (Semi-NH)	Ecological assemblages that have been substantially modified in their composition, balance or function by human activities. [a]	(Stephens, Castro-Morales & Quintana-Ascencio 2012)		
Simulated natural habitat (Simulated-NHs)	A wholly constructed environment, made to resemble a NH.			
Laboratory conditions (LC)	A un-natural environment, variables are clearly defined and controlled.	(Mattana *et al.* 2016)		

[a]= (European Investment Bank 2018)

In LC germination experiments, such as those using incubators, most variables are kept constant (e.g. light intensity, sowing medium, water-availability, timing of diurnal temperature cycle), and one or two are manipulated with a few combinations (e.g. three or four temperatures of diurnal cycles). Researchers select the conditions of variables based on knowledge from NH microclimates, but in reality, these are notoriously difficult to exactly define (e.g. Dinsdale, Dale & Kent 2000). Indeed, important variables may inadvertently be omitted from experimental designs. Interpretation of LC experiments in an ecologically meaningful way is difficult because it will be based on many assumptions, both in selecting variables to test and extrapolating interpretation to NHs - especially if NHs have not been adequately studied.

By contrast, in NH experiments, many, often unknown, variables interplay, one or two of which may be controlled. If researchers were to control all combinations of NH variables in LCs they would soon run out of seeds, time and space. Interpretation of NH experiments depends on dynamically recorded variables that

cannot be well controlled, some even being irrelevant to germination ecology. It goes without saying that NH experiments can only truly be performed in regions where the plant is native, whereas LC experiments can be carried out anywhere with suitable equipment.

Alternatives to NH and LC are semi-natural habitats (Semi-NHs) or simulated natural environments (Simulated-NHs). Examples of Semi-NHs include fields or farm edges, in or close to a species' native region. The term simulated-natural environment (or habitat as used here) was used by Kaeberlein *et al.* (2002) to define an environment of natural seawater and sediment the authors placed in aquariums to culture previously 'uncultivable' marine microorganisms. Such Simulated-NHs are less controlled than LCs, but allow better interpretation of findings and may include important factors that are not well understood or known and so may inadvertently be omitted from LCs. Glass houses, such as those in botanic gardens, are examples of Simulated-NHs, as they mimic NHs and variables are not well under control.

In botanic gardens, living collections are often arranged according to geographic plant communities, each compartment or grouping representing a pseudo or Simulated-NH. These living collections are a valuable resource in studying plant ecology, particularly when NHs are challenging to access (Perez *et al.* 2019). Many botanic gardens also hold seed banks (469 gardens), and carry out seed or spore research (155 gardens) (BGCI 2021). As botanic gardens are biased towards temperate regions in the Northern hemisphere (Mounce, Smith & Brockington 2017), there is opportunity to enhance interpretation of seed germination studies using botanic gardens as Simulated-NHs when it is not possible to do so in native regions (Faraji & Karimi 2020).

Wild banana species (*Musa* L.) are native to tropical and subtropical Asia to the western Pacific (Govaerts & Häkkinen 2006). Their fruit contains many hard darks seeds, 3-7 mm in diameter (Chin 1996). The conditions for germination are not well understood and germination is notoriously inconsistent and often very low (Kallow*et al.* 2020; Panis, Kallow & Janssens 2020; Singh *et al.*2021). LC experiments show a requirement for alternating temperatures (Stotzky & Cox 1962; Kallow *et al.* 2021), but no NH experiments have been executed to interpret this. For instance, is this requirement a gap or depth detection mechanism affected by microclimates? And do species respond differently?

Understanding seed germination ecology of wild bananas is not only of ecological interest, it is also important for global food security. Seed banking crop wild relatives efficiently protects genetic material and makes it available for phenotyping and breeding (Dempewolf *et al.*2017), it is included in UN Sustainable Development Target 2.5 (UN General Assembly 2015). Optimized germination is a vital component of seed bank management and breeding - without it, viability is difficult to monitor and access to plants for research and breeding is constrained (FAO 2014; Batte *et al.* 2019; Amah *et al.* 2020).

In the present study we examined germination responses in a Semi-NH and Simulated-NHs of the two primary crop wild relatives of banana:*Musa acuminata* (subsp. *siamea* N.W. Simmonds, and subsp.*burmanicca* N.W. Simmonds), and *M. balbisiana* Colla (De Langhe *et al.* 2009). Specifically, we aim to answer the following questions that cannot be answered in LCs: (1) What environments stimulate or inhibit *Musa* germination? (2) Are *Musa* seeds dormant, and if so how is this broken in the environment? (3) Can*Musa* seeds remain viable in the soil?

Materials and methods

1. *Semi-natural habitat (nursery, Philippines)*
2. *Plant material*

We collected a bunch (an infructescence) of *Musa balbisiana*(accession GB61996) containing seeds from the field genebank at the National Plant Genetic Resources Laboratory (NPGRL), Institute of Plant Breeding, University of the Philippines, Los Baños. Seeds were extracted by opening fruit and washing seeds in flowing water to remove all pulp. Seeds were then left on a tray in the laboratory to surface dry for seven days prior to sowing.

Table 2. Accessions used for germination experiments in simulated natural environments, V= viability percentage from embryo rescue tests in 2019 and 2020.

Accession	Taxa	Source	Native distribution [b]	Year col
GB61996	*M. balbisiana*	Philippines	Trop. & Subtrop. Asia	2019
bur60	*M. acuminata* subsp. *burmannica*	Guadeloupe	SW. India, China (S. Yunnan) to Indo-China	2014
sia61-63	*M. acuminata* subsp. *siamea*	Guadeloupe	Indo-China to N. Pen. Malaysia	2014
bal106	*M. balbisiana*	China (Hainan)	Trop. & Subtrop. Asia	2017
bal115	*M. balbisiana*	Nigeria	Trop. & Subtrop. Asia	2019

b = Govaerts and Häkkinen, 2006

Solar radiation and substrate

We used the nursery of the NPGRL (latitude 14.153, longitude 121,262), as a Semi-NH for germination testing. We used locations either exposed to solar radiation ('sun') - only lightly shaded in the fine screen house, or without direct exposure to solar radiation ('shade') - in an open-sided cabinet covered at the top also in the screen house. We sowed seeds in plastic trays (100x40x10 cm), using two types of substrate (clay loam soil and fine sand), and covered seeds with 5 mm of substrate. Two replicates of 200 seeds were used for each treatment combination. We recorded the temperature and relative humidity (RH), every 20 minutes for 55 days in sowing locations using data loggers (Tinytag View 2, Gemini Data Loggers, Chichester, UK). Trays were watered daily, emergent seedlings were recorded and removed weekly. The test was concluded after 55 days.

Simulated-natural environment (glass house compartments, Belgium)

Plant material

We studied germination responses in Simulated-NHs in relation to foliage-shading and seed burial-depth using two *Musa* species (total three taxa, Table 2). Seeds were selected from the collection of Bioversity International/KU Leuven (Leuven, Belgium) and were supplied for scientific use with a phytosanitary and origin certificate from China (Hainan), Guadeloupe, and Nigeria. Seeds were from open-pollinated accessions in living collections and were air shipped from source to Leuven as complete bunches, where they were extracted as described above, apart from bal106 which were provided as extracted and cleaned seeds transported to Leuven, Belgium. After ambient drying, seeds were placed in the refrigerator in paper bags, until 2019 when they were sealed in aluminum bags and stored in the refrigerator at approximately 6% moisture content, fresh weight basis. Viability was assessed prior to sowing (both in 2019 and 2020) using ER (method described by Kallow*et al.* 2020).

Foliage-shading and seed burial-depth

We selected a total of six Simulated-NHs for germination tests. Five were in three compartments of Meise Botanic Gardens glass house, Belgium (latitude 50.925, longitude 4.330), and one in the full-ground glass house of KU Leuven, Belgium (latitude 50.860, longitude 4.680). Simulated NHs were selected to represent various heating regimes in different compartments and levels of foliage-shading/exposure to solar radiation (Table 3). All compartments included living banana specimens.

Seeds were sown in square plastic pots (9x9x10 cm), at two burial-depths (1 cm and 7 cm from the surface), using potting compost (Peltracom, composition: 70 % white peat and 30 % black peat; pH: 5.5- 6.5; particle size: 0-10 mm). Two replicates of 30 seeds of each accession were sown in separate pots which were then also buried to be level with soil surface. Data loggers (Tinytag Transit 2 TG4080, Gemini Data Loggers, Chichester, UK) were buried at each location and burial-depth. Loggers were set to record temperatures every 20 minutes. Additionally in 2020, loggers were placed at the soil surface to record light intensity and surface temperature (HOBO Pendant MX2202, Onset, Cape Cod, Massachusetts, US). Germination was

monitored weekly and emergent seedlings were recorded and removed. Seeds were sown in early March 2019, and again in early March 2020s, this is the end of the winter/start of the spring season in Belgium (mean outdoor temperature 6.4°C, climate-data.org). The experiment was concluded in March 2021.

Table 3 . Summary heating regimes of glass house compartments used, temperatures are the thermostat temperature below which artificial heating is instigated.

Compartment name	Institution	Exposure	Day (°C)	Night (°C)
Leuven	KU Leuven	Exposed	15	15
Mabundu	Meise Botanic Gardens	Exposed	25	25
Tropical	Meise Botanic Gardens	Shaded	20	18
Tropical	Meise Botanic Gardens	Exposed	20	18
Spring	Meise Botanic Gardens	Shaded	10	8
Spring	Meise Botanic Gardens	Exposed	10	8 o

Dormancy and stratification in the soil

To assess dormancy and dormancy loss in the soil (stratification), seeds from the two *M. balbisiana* accessions (bal106 and bal115) were incubated at alternating 35°C in the light for 6 hours and 20°C in the dark for 18 hours (based on Stotzky, Cox & Goos 1961; Kallow *et al.* 2021). Additionally, seeds were buried at two of the cooler Simulated-NHs (Spring/Exposed and Leuven/Exposed) in March 2019 and exhumed each month for a total of three months and placed in the incubator conditions described. Seeds were enclosed in small nylon meshed bags and were buried at 7 cm depth in March 2019. Two replicates of 30 seeds were used for each treatment and accession. For incubation, seeds were sown on moist sand (50 g fine sand, 14 ml deionized water) in Petri dishes (9 cm diameter), sealed in plastic bags, at Meise Botanic Garden. Germinated seedlings of these were recorded and removed weekly for two months. Germination was counted as radicle emergence to 2 mm.

Survival in the soil

At the end of the experiment, seeds planted at shaded Simulated-NHs (locations that showed no emergent seedlings in results) were extracted from pots and tested for viability using a tetrazolium chloride staining test or by incubation. Seeds sown in both 2019 and 2020 were used. Pots containing seeds were removed, seeds were separated from compost by sieving under running water. Extracted seeds from the first replicate of each treatment were tested for viability with tetrazolium chloride (TTC) with a maximum of 20 seeds. The TTC tests were carried out on embryos carefully extracted from seeds, using 0.5 % TTC solution buffered to pH 7 (method described by Kallow *et al.* 2020), incubated at 24°C for 24 hours in the dark. Extracted seeds from the second replicate were sowed in Petri dishes on top of moist sterilized potting compost (Peltracom, composition: 70 % white peat and 30 % black peat; pH: 5.5- 6.5; particle size: 0-10 mm) and placed in an incubator at a 24-hour cycled temperature pattern (based on temperature readings from Los Baños, the Philippines - sun exposed site, Fig. 1a). Seeds in the incubator were monitored every two weeks, for a maximum of five weeks. If very few seeds were extracted from the soil for a replicate, TTC tests were prioritized above incubator germination tests.

Data analysis

We calculated summary indices for germination tests using the *GerminaR* package in R (Lozano-Isla, Benites-Alfaro & Pompelli 2019). These included final germination percentage (GRP, %) (Labouriau & Valadares 1983), mean germination time (MGT, days) (Czabator 1962) and synchronization index (SYN, simultaneous germination within a replicate = 1, no overlap between seeds of a replicate = 0) (Primack 1980; Ranal & Santana 2006).

We summarized data logger readings to extract the mean, maximum, minimum, and range (maximum-minimum) during experimental 'cue periods'. In semi-NH this was the whole experimental period (55 days).

In simulated-NHs for each year we summarized data filtered to include readings during 'cue periods' - these were a period prior to the MGT of each replicate, set differently for shallow and deep planting based on the mean MGT for shallow and deep planting (shallow=32 days, deep=39 days). For locations with no germination, we used the first 39 days after sowing. We summarized logger readings for cue periods by calculating mean, maximum, minimum, range temperatures and light intensity readings (in 2020). We removed light intensity readings in the dark (<40 lux), to account for changes in day/night length during seasons and did not calculate minimum light intensity as it would be the same in any site.

We then carried out redundancy analysis (RDA) of summarized logger data in cue periods, scaled to unit variance, against a corresponding matrix of factorial variables (species, compartment, exposure, depth) using the*vegan* R package (Oksanen *et al.* 2019). The minimum adequate RDA was found by comparison of Akaike Information Criterion (AIC), by adding variables to the minimum model.

Final germination, for each experiment, was assessed using counts of germinated seeds at the end of experiments against seeds that did not germinate, thus accounting for sample size variance. Sample sizes were adjusted in the analysis to only include viable seeds, estimated from ER results of that year, using the formula:

$$adjusted\ sample = seeds\ sown\ x\ viability\ of\ year\ sown$$

These data were used in generalized linear modelling (GLMs) of binomial data with logit link function. If overdispersion was present in binomial GLMs we used quasibinomial error structure. Minimum adequate models (MAM) were produced by removing variables from maximum models after comparisons with ANOVA and X^2 test. Estimated marginal means were made from models and used for post-hoc analysis using the *emmeans* R package (Lenth 2020). Additionally, we used GLMs for factorial variables on MGT and SYN using gamma error structure. We assessed the effect of environmental variables in cue periods on binomial germination outcomes using GLMs as described. Following this we tested variables for breakpoints to assess temperature thresholds or optimums. We did this on GLMs produced for each microclimate variable separately by using the algorithm with bootstrapping in the*segmented* R package (Muggeo 2017). We used starting points estimated by plotting GRP against microclimate variables and trend lines using the non-linear regression method Loess.

We compared viability of seed survival of seeds exhumed after 1-2 years in the soil from the incubation test, TTC test, and maximum achieved from Simulated-NHs, against the original ER viability. We again used a binomial GLM for this, as described, and post-hoc contrasts against original ER with Dunnet tests. All analysis was performed in R (R Core Team 2020).

Results

1. *Effects of exposure to solar radiation and substrate on seed germination*

In the semi-NH exposure to direct solar radiation (sun) rather than shade, significantly increased germination rates (z=20.963, p<0.001, Fig. 1a). There was no significant effect of substrate on germination outcome according to the GLM. Final germination percentage in the sun was 72% (soil), 71% (sand) whereas in the shade it was 19% (soil), 12% (sand).

The MGT in the shade was 22±2 days (mean, standard deviation, used hereafter), whereas in the sun it was slightly faster germinating in 18±2 days, albeit only few seeds germinated in the shade. Synchronization index in the sun was 0.25±0.10 and in the shade 0.27±0.07.

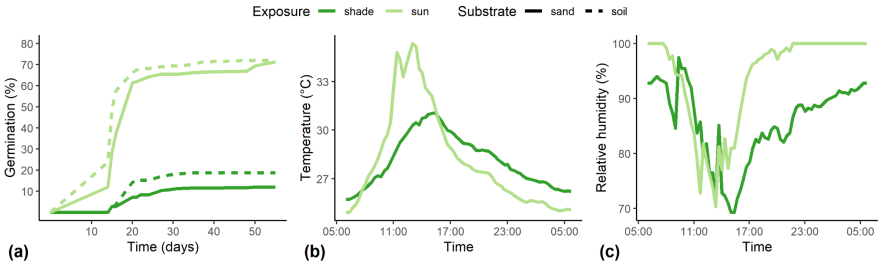

Fig. 1 (a) Cumulative germination of *Musa balbisiana*seeds sown in the semi-natural habitat (nursery, Los Baños, the Philippines), seeds were sown in the sun and shade, and in sand and soil (n=200, 2 replicates, mean values shown); (b) typical diurnal temperature and (c) relative humidity profiles logged at sowing site during germination test (Los Baños, the Philippines, 16th November 2019).

Mean daily temperature in sun and shaded exposure was identical, but standard deviation was approximately 1°C greater in the sun (27.3±2.8°C sun, 27.3±1.9°C shade, Fig. 1b, Table 4). Most notably, this relates to warmer maximum temperatures in the day of around 4°C in the sun. Mean diurnal range in the sun was therefore also greater (8.9°C sun, 5.1°C shade). Humidity in the sun and the shade was broadly similar, ranging diurnally between 75 and 100% RH (Fig. 1c), a function of temperature.

Table 4. Summaries of the daily temperature and humidity recordings in the sun and shade at the location of the seed germination experiments. Values in brackets are standard deviations of means.

	Sun	Sun	Sun	Sun	Shade	Shad
	Temperature (°C)	Temperature (°C)	Relative humidity (%)	Relative humidity (%)	Temperature (°C)	Tem
Daily	Night	Day	Night	Day	Night	Day
Mean	24.5 (±0.8)	33.4 (±3.6)	81.3(±16.6)	100 (±0.1)	25.2 (±0.7)	30.3
Max	26.2	42.2	100	100	26.7	38.5
Min	23.0	24.7	10.1	99.6	23.6	25.8

Effects of foliage-shading and seed burial-depth on seed germination

Microclimate in simulated natural habitats

Cue period microclimate variables were constrained by the factors room and exposure in the minimum adequate RDA, and notably not by seed burial-depth or species (r^2=0.88, Fig. 2). Exposure had the greatest influence on the microclimate RDA (df=1, F=262.15, p<0.001), room had more variance (df=3, F=144. P<0.001). Unsurprisingly, compartments with higher thermostat temperature thresholds (Table 3) were clustered with higher mean and minimum temperatures. These clusters did not include variables relating to extremes i.e., maximum temperatures, temperature ranges and soil surface values (for temperature and light). These were clustered separately and correspond to exposed microclimates. Shaded microclimates varied less than exposed, hence the different shapes of ellipses in Fig. 2.

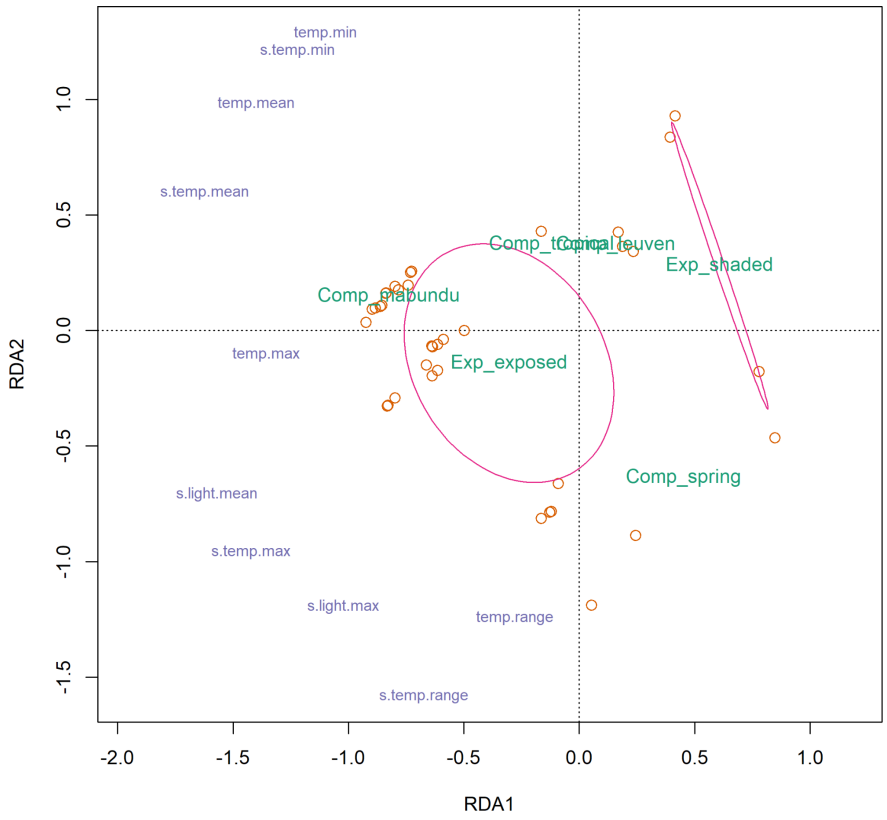

Fig. 2 Redundancy analysis ordination of microclimates used as simulated natural habitats in glass houses for seed germination experiments (r^2=0.88), ellipses are exposed and shaded sites (confidence limit= 0.95), green text is explanatory factors (Comp=compartment, Exp=level of exposure to sun), blue text are microclimate variables achieved from data loggers at site (s=from data logger at soil surface, temp=temperature).

Shaded microclimates obviously received less light intensity (731±240 lux shaded, 4491±1621 lux exposed) (Fig. S1) and had a lower maximum temperature (25.8±1.0°C shaded, 40.7±7.1°C). In the soil, the maximum temperature, again, was less at shaded sites compared to exposed sites (19.5±2.2°C shaded, 27.1±4.6°C exposed). The relationship between light intensity and temperature at the soil surface was asymptotic, above 2000 lux there was very little increase in temperature (Fig. S2).

Effects of microclimate factors on seed germination

In simulated-NHs, no seeds germinated in any shaded microclimates. These were then excluded from statistical analysis. Compartment and depth remained in the MAM, species and year were excluded after testing (Fig. 3a). Seeds had significantly higher probability of germinating in the two compartments that have a higher heating regime (compartments Mabundu , z=4.531, p<0.001, and Tropical z=6.586, p<0.001). Across all rooms, shallower buried seeds were more likely to germinate (z=6.491, p<0.001).

Seedling emergence happened sooner after sowing in the compartments with warmer heating regimes (Mabundu, t=8.557, p<0.001 and Tropical 6.805, p=<0.001, Fig. 3b). Predictably, shallow buried seeds at 1 cm emerged quicker than those buried at 7 cm (t=2.657, p=0.010). There was no effect of species on time to germination (seedling emergence), so this was excluded from the model, leaving compartment and burial-depth.

Synchronization was significantly greater in the cooler compartments (Spring and Leuven) for *M. balbisiana* (Fig. 3c), particularly in the pairwise contrast between Leuven and Mabundu (z=3.110, p=0.010).

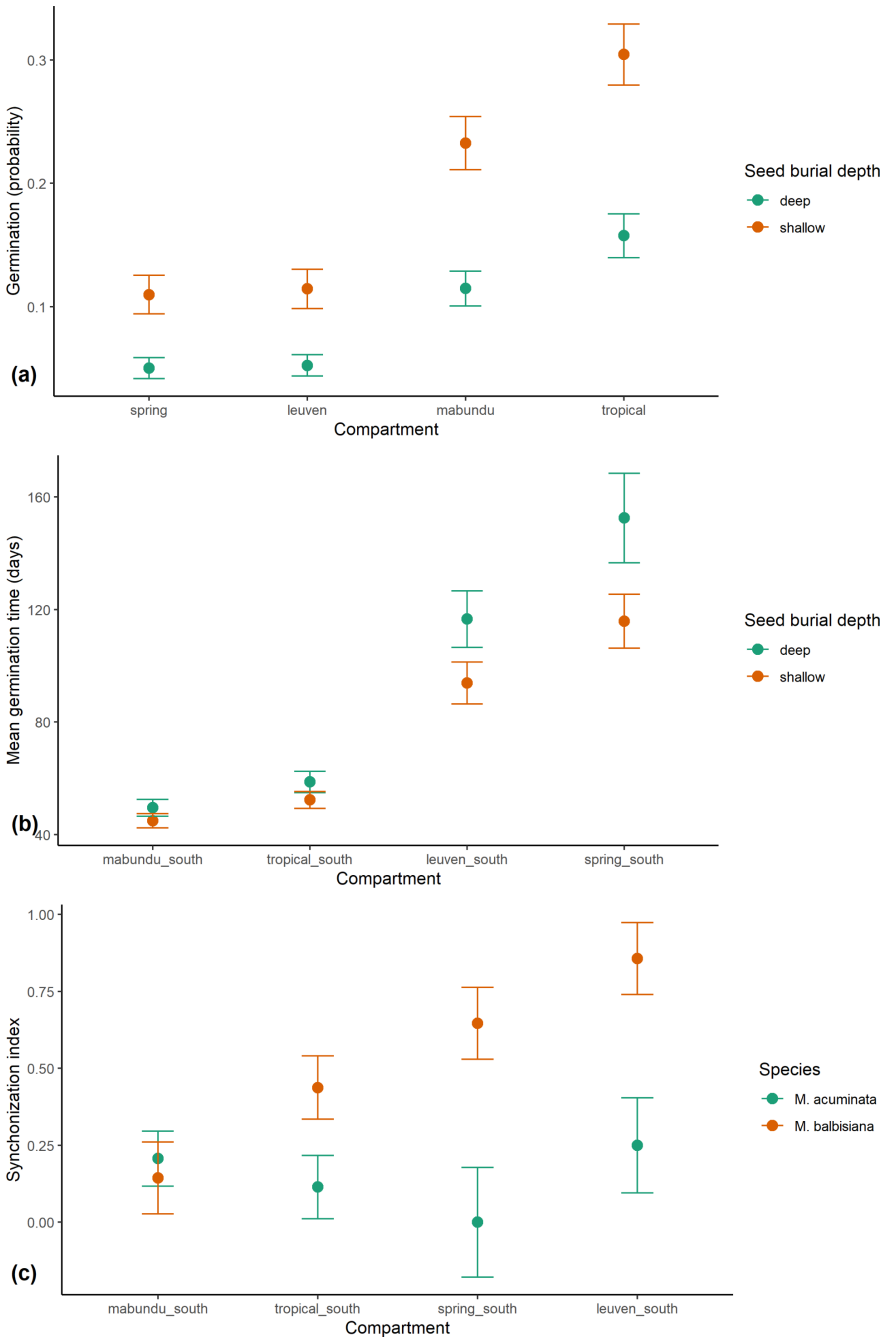

Fig. 3 Estimated marginal means of minimal adequate GLMs on germination of *Musa* seeds buried in Simulated-NHs in Belgium (a) Binomial germination outcome; (b) mean germination time; (c) synchronization index.

Effects of microclimate variables on seed germination

The effect of each microclimate variable on final germination percentage was visualized (Fig. S3). These were then used to estimate breakpoints on GLMs produced from binomial germination outcomes (Fig. 4). Results of this show temperatures (at seed burial level) operate a threshold mechanism, above which germination

increases linearly (on the logit scale), within the limits of temperatures achieved in simulated-NHs. Therefore, for germination to occur mean soil temperature needs to be above 19°C, maximum temperature above 23°C, minimum temperature has much less of an effect. At the soil surface, the effect of mean temperature is positively linear to an optimal 24°C after which germination is reduced. The effect of maximum temperature is positive above a threshold of 28°C. Germination increases with soil surface minimum temperature to 15°C, then the positive slope is less steep. Soil warming relates to light intensity asymptotically (as described above); germination increased above 1076 lux (mean). In relation to maximum light intensity, germination increased to a breakpoint at 54000 lux, after which the effect on germination is negative. Comparisons of AICs of these models (Table S1) show at seed burial level the maximum temperature is the best fit; at soil surface level mean temperature is the best fit, and mean light intensity is a better fit than the maximum value during cue periods.

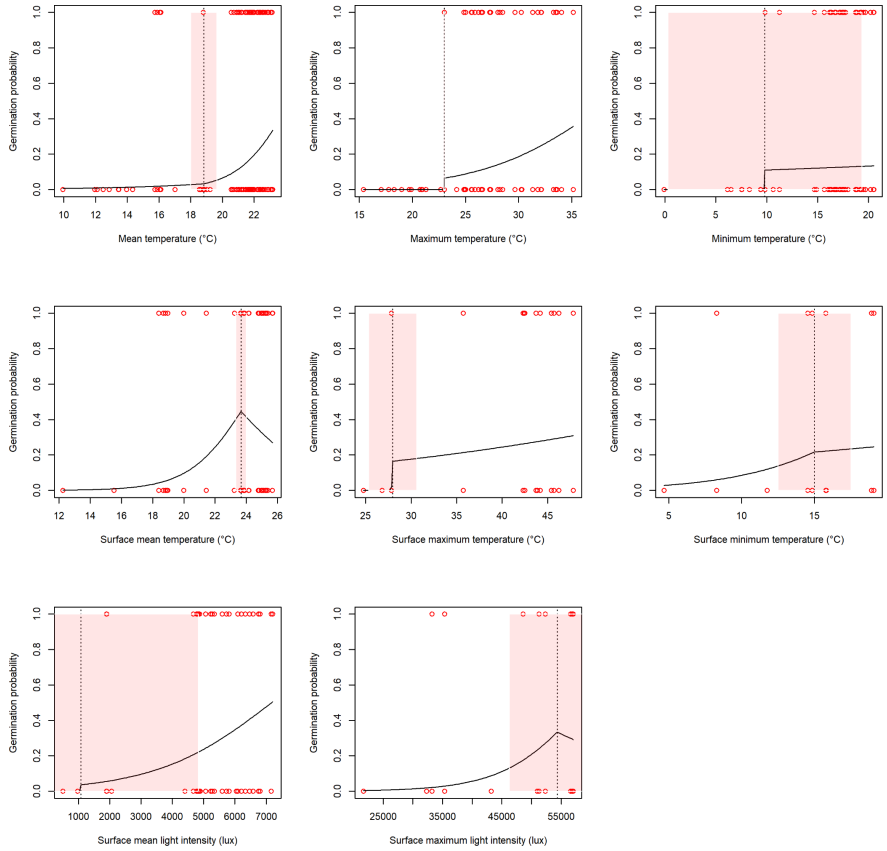

Fig. 4 Segmented GLMs of binomial germination outcomes back-transformed to the response probability scale as response to microclimate variables in simulated-NHs, dashed lines are breakpoints and pink shading is standard error (0.95), residuals are red circles.

Survival and dormancy loss in the soil

Overall, 40% of seeds that were buried in the soil for two years and 68% for one year were successfully exhumed from the soil. The probability of finding seeds was modelled against year, burial-depth, and accession (Fig. 5a). Seed burial-depth was the most important factor, deep-buried seeds were more likely to be successfully exhumed ($z=16.722$, $p<0.001$), followed by year ($z=13.379$, <0.001). Surprisingly, seeds with low viability when sown had high probability of being exhumed (bur60 $z=7.242$, $p<0.001$, sia61 $z=7.025$, $p<0.001$).

Viability of exhumed seeds were tested by TTC and incubation. Viability tests from these, original ER and

maximum in Simulated-NHs, was modelled in a GLM. In contrast to the results of the above, accession was excluded from the MAM, year remained. Contrasts were made per year against original ER. Viability from the incubator test was not lost during one year in the soil. After two years in the soil, viability was reduced in both incubator and TTC tests. The TTC test consistently underestimated viability.

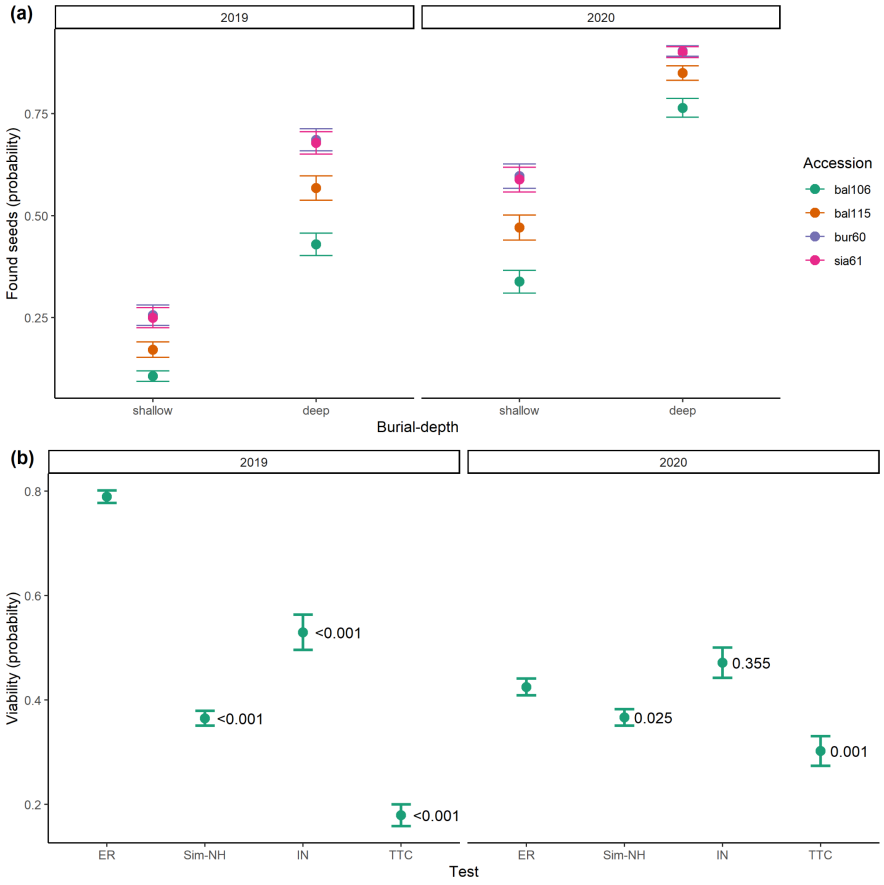

Fig. 5 Seed survival in the soil; (a) Probability of finding seeds in 2021 that were sown in 2019 and 2020 according to burial-depth and accession; (b) viability of seeds found in 2021 using a germination test in an incubator (IN) and the tetrazolium chloride test (TTC), with reference to viability at the start of the experiment using embryo rescue techniques (ER), and maximum germination achieved in a Simulated-NH; p values show contrasts (Dunnett test) against original viability (ER) for each year.

Seeds that were removed from storage and incubated under suitable conditions (20/35°C), displayed dormancy i.e., they did not germinate at all. However, if they were buried in the soil at 7 cm for up to three months, germination significantly increased to probability 0.4 (t=2.518, p=0.021, Fig. 6).

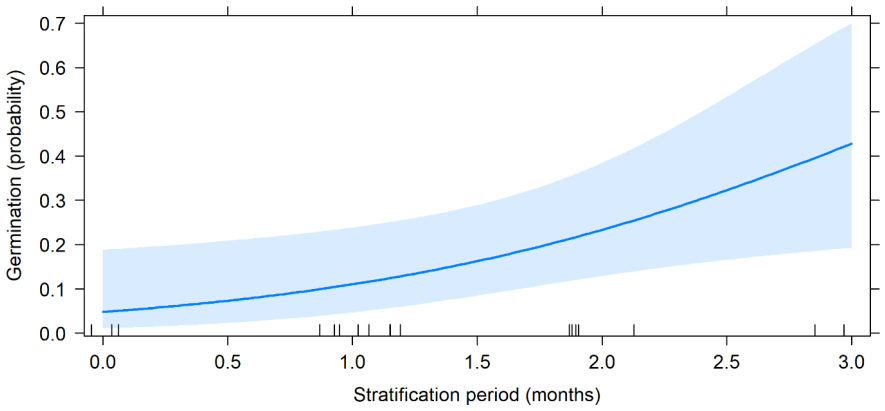

Fig. 6 Germination probability in an incubator (at 35/20 °C, light/dark, 18/6 hours cycled) of seeds buried and then exhumed from the soil for up to three months. Seeds were buried in the exposed Spring compartment.

Discussion

1. *Use of semi-natural and simulated habitats for germination ecology experiments*

In the present study we used a Semi-NH from the native range of *M. balbisiana* , and several Simulated-NHs in glass houses located in a temperate region, to examine wild banana seed germination ecology. We established that with such approximations of NHs, it is possible to link germination responses to ecological factors such as foliar-shading and burial-depth. Hence with this approach we overcame limitations when access to experimental NHs was not possible, this allowed for greater ecological interpretation than with LCs alone.

Temperature

We found that *Musa* seed germination is stimulated by exposure to the sun. It was the maximum part of the temperature fluctuation, in our results, that is most closely associated with germination. Above the threshold of 23°C, germination increased to a maximum at 35°C (in Simulated-NHs and 42°C in Semi-NHs) in exposed conditions. Our findings are broadly consistent with previous LC results, where optimal maximal and minimal temperature for germination were diurnal cycles of 35/18-20°C respectively, for both *M. acuminata* and *M. balbisiana* (Stotzky & Cox 1962; Kallow *et al.* 2021). Interactions between the elements of warming and cooling cycles play an important role in simulating germination.

Light

One might think that as germination responses were directly associated with soil exposure to sun and, in simulated-NHs, light intensity, germination is stimulated by light. Additionally, we also found that seeds germinated to a greater extent from 1 cm compared to 7 cm. However, light waves cannot usually penetrate the soil to greater than 4-5 mm depth (depending on soil moisture and particle size), and not to any amount that can illicit germination responses to light sensitive seeds (Woolley & Stoller 1978; Tester & Morris 1987), but in our experiment, seeds germinated from a depth of 7 cm. We therefore infer that whilst *Musa* seed germination may be correlated with factors associated with light (light intensity, shallow burial) theses are correlations rather than causal, and it is temperature that regulates germination.

Gap detection

Musa seed germination in response to sun exposure demonstrates adaptation to detect suitable niches for seedling establishment following disturbance in forest NHs. Conversely, inhibition of germination in shade is also an adaptation for seedling survival (Kos & Poschlod 2007; Poschlod *et al.* 2013). *Musa* germination

responses were sensitive to sun/shade even when microclimates were very similar. For instance, mean temperatures were the same and range differed by only a few degrees in sun and shaded semi-NHs. Germination is therefore finely tuned to respond to microclimate such as that which would occur when a forest gap is formed (Pearson *et al.* 2002; Pearson *et al.* 2003). The effect of forest disturbance on temperature dynamics was studied by Harwick *et al* . (2015). The authors measured soil (10 cm depth) and air temperature (1.5m height) at three levels of forest disturbance in Borneo. Diurnal temperatures in oil palm plantations (formerly forested) were around 7°C greater at the hottest part of the cycle compared to old growth forests and soil temperatures were around 3 °C warmer at this point and around 1 °C cooler in the night; these were similar to our results in semi-NHs. Temperatures in logged forests were somewhat in between these two, but more similar to old growth forest. One could imagine that even in small forest gaps, microclimates could therefore also vary considerably (Pearson *et al.* 2002). These responses are in line with adaptations of other disturbance-adapted species that also require alternating temperature cycles rather than constant temperatures to germinate (Vázquez-Yanes & Orozco-Segovia 1982; Vazquez-Yanes & Orozco-Segovia 1993; Pearson *et al.* 2002; Seiwa *et al.*2009).

Seed burial-depth

When seeds were in the shade, burial-depth made no difference to germination, they did not germinate irrespective of burial-depth. In exposed sites, shallow buried seeds were more likely to germinate than deeply buried seeds. This was because temperature dynamics are likely buffered by burial depth. For some species, sensitivity to alternating temperatures is an adaptation to detect burial depth (Thompson, Grime & Mason 1977; Thompson & Grime 1983). Pearson et al. (2002) found, for large-seeded species, diurnal temperature sensitivity was more likely to related to forest gap size than seed burial-depth. This was also the case for large-seeded *Musa* , in that exposure was by far the most significant factor in simulated-NHs, and burial-depth was only secondary. For small seeds it is important to detect burial-depth as seedlings must reach the surface with small endosperm reserves; for larger seeds with greater nutrient reserves this is less of a limiting factor for survival.

Survival and dormancy loss in the soil

We found seeds can persist and remain viable buried in the soil for at least two years. In fact, there was no loss of viability after one year, there was however loss of actual seeds. Seed loss was more pronounced with shallow burial, suggesting it is the result of predation or perhaps splashing during watering, rather than decomposition. This is also supported by the fact that accessions with less viable seeds were more likely to be found i.e., seeds were not lost by decomposition of dead seeds.

For seeds adapted to disturbance, seed persistence in the soil seed bank is important. *Musa* clearly invest considerably in seed coat defenses (Graven *et al.* 1996), to survive the intense pressure present in the soil community (Dalling *et al.* 2011).

Not only do seeds persist in the soil, but dormancy is reduced during this process. In our results, when seeds were stratified for three months, or when they were in the soil for a year, germination increased. A stratification requirement is in keeping with results from our previous study (Kallow *et al.* 2021). Although, stratification was not required for freshly extracted *M. balbisiana* seeds in Semi-NHs, implying drying induces secondary dormancy in *Musa*seeds, as proposed by Chin (1996).

There was greater germination synchronization in cooler Simulated-NHs as seeds responded to threshold temperatures when sun was stronger during the summer. When temperatures were consistently warmer, synchronicity was reduced - this may again be a disturbance adaptation. We found this response more evident in *M. balbisiana* than in *M. acuminata,* suggesting it may also relate to seasonality as *M. balbisiana* has a large distribution that includes subtropical seasonal climates (Mertens *et al.* 2021).

Conclusions

Studying germination ecology has intrinsic challenges, possibly the biggest being access to seeds and experimental set-ups in suitable conditions and timeframes. In the present study we demonstrate an approach for dealing with such difficulties in studying tropical seed germination ecology, which is a challenge when researchers are outside of a plant's native region. Using Semi-NHs and Simulated-NHs we found: (1) foliage-shading inhibits germination of non-dormant seeds, and exposure to sun stimulates germination; this response is most closely associated with maximum temperature variation found under direct sunlight; this effect is marginally buffered by deep burial in the soil; (2) freshly extracted seeds are non-dormant, but stored seeds lose their dormancy during burial in the soil; (3) *Musa* seeds remain viable in the soil for at least a year without any loss in viability. Thus, wild banana species are well adapted to exploit canopy gaps following disturbance.

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Author contributions

SK: conceptualization, methodology, software, formal analysis, investigation, data curation, writing-original draft, writing-review and editing, visualization; KQ: investigation; BP: conceptualization, methodology, resources, writing-review and editing, supervision, funding acquisition; SBJ: conceptualization, methodology, resources, writing-review and editing, supervision, funding acquisition; JD: writing-review and editing, supervision, funding acquisition; LG: resources; RS: writing-review and editing, supervision; FV: conceptualization, methodology, resources, writing-review and editing.

Conflict of interest

The authors declare that there is no conflict of interest associated with this article and research.

Data availability

All data available at Kallow, Simon (2021): Using semi-natural and simulated habitats for seed germination ecology. figshare. Dataset. https://doi.org/10.6084/m9.figshare.14884470.v1

Supplementary figures and tables

Fig. S1. Average and standard deviations of temperature and light intensity during cue periods of exposed and shaded environments, temperatures are degC, light intensity is lux.

Fig. S2. Light intensity and temperature measured at simulated natural environments in glass houses during germination tests; asymptotic non-linear regression shown (residual standard error 4.119 on 417,661 degrees of freedom).

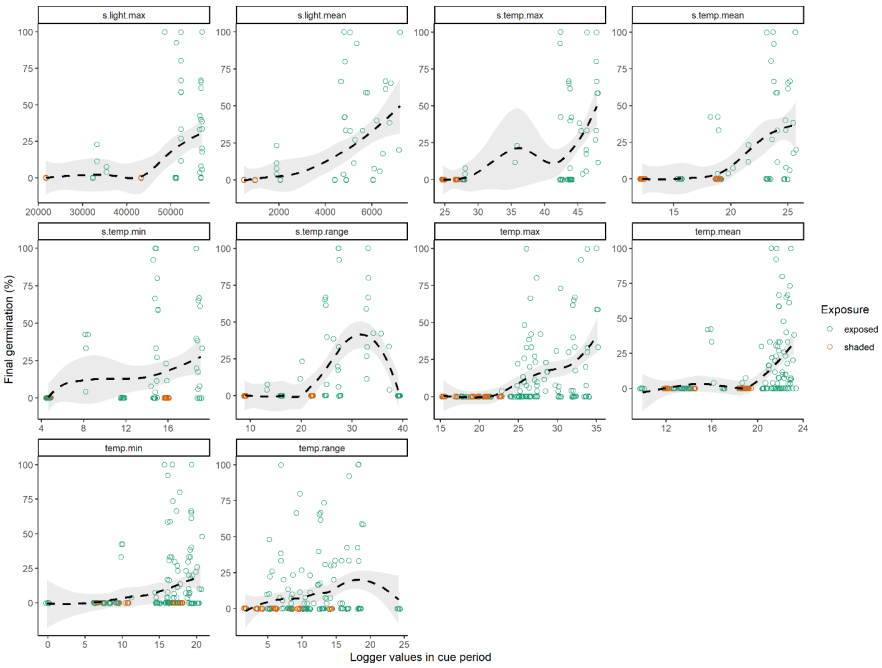

Fig. S3. Final germination percentages and microclimate variables from simulated-NHs, dashed trend lines plotted with Loess non-parameter regression, shaded area is 95% standard error, light variables are in lux, temperature are °C, s=value from logger at the soil surface, otherwise they are at seed burial depth.

Table S1. Model fits of germination outcome in response to microclimate variables in simulated-NHs in segmented GLMs.

Variable	AIC	Residual deviance	df
temp.mean	1749	1741	3388
temp.max	1612	1604	3388
temp.min	1890	1882	3388
s.temp.mean	799	791	1220
s.temp.max	817	810	1220

Variable	AIC	Residual deviance	df
s.temp.min	961	953	1220
s.lightemp.mean	803	796	1220
s.lightemp.max	887	880	1220

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