

Increasing the germination envelope under water stress improves seedling emergence in two dominant grass species across different pulse rainfall events

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Summary

1. Demographic recruitment processes, such as seed germination and seedling emergence, are critical transitional phases to the re-establishment of degraded plant populations, but often fail due to rainfall not supporting plant requirements. Using species from the widespread arid Australian perennial grass genus *Triodia*, we investigated the interactions of seeds in different dormancy states and their functional germination envelope in response to water stress after simulated pulse rainfall events.

2. Seed dormancy was alleviated in *Triodia* species to varying degrees by wet/dry cycling or by removing floret structures from seeds. The seeds were then exposed to different rainfall frequency and quantity events mimicking the 25th, median, 75th and 95th percentile rainfall events found in natural habitats for the study species in the north-west Australian arid zone.

3. Under 95th percentile rainfall conditions recruitment was highest, but still limited to 35% germination and 10% emergence of cleaned seeds (i.e. the least dormant state evaluated). This was related to the functional germination envelope as indicated by more negative base water potential thresholds (Ψ_{b50}) for cleaned seeds (≥ -0.33 MPa) compared to intact florets (≥ -0.26 MPa). As a result, the maximum cumulative time where soil water potentials were optimal for germination ($\Psi_{soil} \geq \Psi_{b50}$) was 1.6–2.6 times longer for cleaned seeds in large frequent rainfall events when compared to intact florets. Furthermore, seed dormancy, that usually prolongs seed survival, was linked to a short-term reduction in seed viability, which may further reduce recruitment rates.

4. *Synthesis and applications.* Our findings indicate that large frequent rainfall events raised soil water potentials above critical thresholds for germination and are important for successful plant establishment. If recruitment bottlenecks are a result of seed dormancy and variable rainfall for arid grass species, then this study shows benefits for alleviating seed dormancy prior to seeding in restoration sites, as this increases the environmental envelope for germination.

Key-words: arid zone, grasslands, plant demography, recruitment bottleneck, rehabilitation, seed dormancy, soil water potential, *Triodia*, vegetation restoration

Introduction

Plant recruitment events in arid ecosystems are often infrequent due to erratic patterns and limited quantities

of significant rainfall (Reynolds, Kemp & Tenhunen 2000), leading to reduced plant recovery rates in anthropogenically altered landscapes. (Millennium Ecosystem Assessment 2005). Furthermore, once degraded, recruitment failure in arid ecosystems rapidly leads to desertification, now impacting c. 250 million people world-wide, and likely to increase above the current degradation

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rate of 12 Mha per annum in response to changing climate trends (IUCN 2011). With 60% of arid ecosystem vegetation comprising grasslands (White *et al.* 2000), understanding recruitment dynamics of grasses in undisturbed and/or degraded situations, and ways to facilitate these processes, is therefore recognised as a global priority (UNCCD 2012). Although global restoration efforts have intensified over the last decade, managers of degraded arid landscapes are often challenged by seed-based recruitment failure to achieve restoration goals.

The re-establishment of viable plant populations following degradation in arid environments largely depends on the transition of a sown seed reaching reproductive adult maturity (James *et al.* 2013). However, current seeding success is typically <10% (Chambers 2000; James, Svejcar & Rinella 2011; Wagner *et al.* 2011). This failure has been associated with four stages and processes of recruitment: (i) seed dormancy, (ii) germination, (iii) emergence and (iv) plant survival (Merritt *et al.* 2007; James, Svejcar & Rinella 2011; Larson *et al.* 2015). To facilitate greater grass recruitment success, the identification of seed dormancy and moisture availability interactions that drive seedling emergence is the first component of the system that needs to be understood.

Seed dormancy is prevalent in over 80% of arid-zone species (Baskin & Baskin 2014) and failure to alleviate seed dormancy may lead to poor germination even during favourable rainfall seasons (Merritt *et al.* 2007). From natural systems, seed germination in arid ecosystems is restricted to a small recruitment window (Baskin & Baskin 2014) constrained to moisture conditions when $\Psi_{\text{soil}} \geq \Psi_{\text{b50}}$ [where Ψ_{b50} defines a minimum water potential threshold for germination (Bradford 2002)]. In arid systems, this generally occurs in response to large rainfall events. However, the condition when $\Psi_{\text{soil}} \geq \Psi_{\text{b50}}$ that typically defines the physical environmental envelope for germination is different between dormant and non-dormant seed populations (Bradford 2002; Finch-Savage & Leubner-Metzger 2006). Dormant seed populations characteristically show a restricted environmental envelope for germination, while with the release of seed dormancy, the environmental envelope for germination widens into more negative water potentials (Bradford 2002).

In grasses, seed dormancy is often a combination of two separate mechanisms acting on the embryo: (i) mechanical restriction imposed by the floret and (ii) a physiological barrier that acts by limiting the growth capability of the embryo (Baskin & Baskin 2014). Under laboratory conditions, physical removal (Ma *et al.* 2010; Erickson *et al.* 2016) or chemical abrasion (Stevens *et al.* 2015) has proven successful in overcoming this mechanical restriction, while after-ripening under controlled humidity conditions have successfully replicated natural physiological dormancy alleviation (Erickson *et al.* 2016). Cycling dormant seeds through a series of wetting and drying

events has also been highly successful (Hoyle *et al.* 2008). While seed dormancy state varies in natural soil seed banks within a seed population and between species (Baskin & Baskin 2014), understanding how seeds at different dormancy states drive recruitment processes during periods of rainfall will provide a critical insight into vegetation re-establishment dynamics.

Although arid-adapted grass species have mechanisms to cope with periods of prolonged drought or water deficit (e.g. Grigg, Veneklaas & Lambers 2008; Jeffrey, Jesse & Clint 2012), early seed and seedling life stages are likely to be more vulnerable to soil water deficits, which may impact on their recruitment. For example, studies by James, Svejcar & Rinella (2011) indicated that between seed and adult plant life stages, the majority of recruitment failure occurs during initial germination and establishment phases, while higher survival probabilities were generally observed after plants were established. This is in part explained by the functional traits in adult plants enabling resource acquisition and utilisation, with these traits not yet established during early developmental phases (Lambers, Chapin & Pons 2008; Bateman *et al.* 2016). With a recent seed and seedling trait framework indicating that the bulk of recruitment (>90%) is explained by germination and emergence probability (Larson *et al.* 2015), traits associated with germination and emergence may be the dominant influence on plant establishment during restoration. Understanding seed dormancy and moisture availability interactions may therefore be crucial predictor for early recruitment success.

This study aimed to understand how seed dormancy and its interaction with soil moisture availability drive grass recruitment in arid systems. Using *Triodia*, a dominant grass of Australian arid ecosystems [covering 1/3 (c. 1.8 Mkm²) of the Australian continent (Lazarides 1997)], as the model genus three hypotheses were tested: (i) overall germination and emergence would be limited under field conditions due to seed dormancy and limited water availability and that widening the germination envelope by decreasing dormancy would result in higher germination and emergence at lower soil water potentials and (ii) the frequency of rainfall events would trigger recruitment events only where a base soil water potential (Ψ_{b50}) is achieved to sustain germination and emergence. *Triodia* species are recognised to possess 'hard' and 'soft' species, based on different leaf anatomical features (Lazarides 1997), resulting in different physiological function in adult plants (Grigg, Veneklaas & Lambers 2008). As a result, hard species are more commonly associated with drier landscapes (Lazarides 1997), it is expected that (iii) this would reflect an advantage for lower water availabilities from rainfall for germination and emergence of hard species. The outcomes of this study will show how seed dormancy/germination and emergence contribute to an improved understanding in recruitment dynamics for arid-zone grassland restoration.

Materials and methods

SPECIES SELECTION

This study tested two functionally different keystone species of *Triodia* from the Pilbara region of Western Australia: *Triodia epactia* S.W.L. Jacobs and *Triodia wiseana* C.A. Gardner, so-called soft and hard-leaved species, respectively. Although *T. epactia* and *T. wiseana* co-occur in some parts of the landscape, *T. epactia* populations more commonly occur in the wetter coastal areas of the northwest, and *T. wiseana* commonly occurs in the arid interior of the Pilbara (Lazarides 1997). *Triodia epactia* and *T. wiseana* seed collections were sourced in March 2011, from Nindethana Seed Service and Native Bushland Seed Supply, and maintained in an air-conditioned (c. 25 °C/50% RH) room until supplied for experimental purposes in September 2011. The seed collections were then transferred to a cool (15 °C), dry (15% RH) storage facility. Prior to experimental use, florets were cleaned to ensure each floret contained one seed, whereby filled florets were separated from unfilled florets with a vacuum-aspirator ('Zig Zag' Selecta; Machinefabriek BV, Enkhuizen, The Netherlands). Floret fill was then confirmed using an X-ray (Faxitron MX-20 X-ray cabinet, Tucson, AZ, USA) set to a single exposure time of 10 s and 22 kV and was considered filled, when a seed was visible, and without any obvious damage or abnormalities to the test. Any florets with abnormalities were removed from the processed batches. A final floret fill of 99% was achieved and deemed synonymous with 99% viability.

CREATING DIFFERENT DORMANCY STATES IN TRIODIA SEEDS

To understand the influence of seed dormancy on the germination potential of *Triodia* material, a gradient of different dormancy states was established: untreated and intact florets (most dormant); florets exposed to wet/dry cycling treatments (dormancy alleviated and floret structures intact); or cleaned to pure seeds (dormancy alleviated and floret structures removed). In the wet/dry cycling treatment, seeds retained within the floret were exposed to four wet/dry cycles each consisting of a controlled 2-day 'wet' hydration on 0.6% (w/v) water-agar plates and followed by a 12-day 'dry' exposure after placement in 50% RH. Storage conditions of 50% RH were achieved by placing florets in an airtight 270 × 270 × 130 mm polycarbonate electrical enclosure box suspended over a LiCl solution (370 g L⁻¹; NHP Fibox, Richmond, Vic., Australia). The wet/dry cycles were conducted in an incubator maintained at constant 35 °C [12-h alternating light/dark, photosynthetic photon flux density (PPFD) = 45 µmol m⁻² s⁻¹] (Contherm Scientific Limited, Wellington, New Zealand). For the cleaned seed treatment, seeds were removed from the floret by carefully rubbing florets through a 1.4-mm sieve to release the seeds from the floret structures. After arriving at final dormancy states, seed viability assessments were undertaken prior to all subsequent laboratory and field experiments. Viability was initially examined under a dissecting microscope to remove floret or seed units showing any abnormalities, followed by a cut test on imbibed intact floret or seed units. From the cut test, viable proportions were defined when intact florets (untreated; or wet/dry cycled) or cleaned seeds exhibited a firm, fresh and intact endosperm with a white embryo. Following manipulation of seed dormancy,

intact florets and seeds were stored at 15% RH at 15 °C for 2 weeks prior to the field trial.

IMPLICATIONS OF SEED DORMANCY STATE ON GERMINATION RESPONSE UNDER WATER STRESS

Before the field trial, untreated florets, wet/dry cycled florets and cleaned seeds were examined under laboratory conditions to quantify germination performance under optimal and water stress conditions. Intact florets or seeds were plated on filter blotters in Petri dishes that were hydrated with different polyethylene glycol (PEG) solutions (PEG-8000; Sigma-Aldrich Pty. Ltd., Sydney, NSW, Australia) simulating 0 MPa (optimal water conditions, no PEG added), -0.01, -0.1, -0.2, -0.3, -0.4, -0.6, -0.8, -1.0 or -1.5 MPa as described in Michel (1983) for 30 °C, and validated with a dew point psychrometer (WP4C Dew Point Potential Meter; Decagon Devices, Inc., Pullman, WA, USA). Germination evaluated at constant 30 °C (12 h alternating light/dark, PPFD = 45 µmol m⁻² s⁻¹) incubator conditions for 30 day to satisfy dormancy criterion according to Baskin & Baskin (2014). Germination was assessed daily and defined as radicle emergence greater than one-third of the floret or seed coat length. After testing, ungerminated florets or seeds were cut tested to determine dead and viable proportions. All dormancy states had batch viability between 96–99% (see Appendix S1, Supporting Information).

INTERACTIONS OF RAINFALL DYNAMICS ON TRIODIA GERMINATION AND EMERGENCE PATTERNS

Soil moisture – rainfall frequency and quantity manipulations

A field trial in Newman (23°21'38.24"S, 119°42'52.14"E), Western Australia, was installed at the beginning of the summer wet season in December, 2012. The study area was considered to be semi-arid, with hot days (max 40 °C) and warm nights (min 25 °C) (BOM 2016). Soil moisture was modified by the application across ten simulated rainfall combinations. Rainfall thresholds of 12, 24, 48 and 96 mm, subsequently mimicking the daily 25th, median, 75th and 95th percentile rainfall events during the wet season in the Pilbara (BOM 2016), were applied to the trial area. Rainfall frequency (repeat applications providing the same overall total) was selected to test repeat occurrences of these rainfall quantities (e.g. 96 mm – 1 × 96 mm, 2 × 48 mm or 4 × 24 mm; 48 mm – 1 × 48 mm, 2 × 24 mm or 4 × 12 mm; 24 mm – 1 × 24 mm or 2 × 12 mm). The delivery of the rainfall combinations was conducted over 7 days, with the single frequency event treatments occurring on day 1, followed by the 2× and 4× frequency events repeated every 2 days after the commencement of the trial.

Field trial design, germination bag burials and seedling emergence lines

The trial area consisted of ten 35-m² sites receiving different rainfall scenarios that were irrigated through sprinklers at a 10 mm h⁻¹ rate. A buffer area of 2 m was created between each site, to minimise overlapping of irrigation. In each site, five replicate 1.5-m² plots spaced 0.75 m apart were cleared of larger rock fragments and debris to homogenise soils across the trial area.

In each plot, 215 untreated florets, wet/dry cycled florets and cleaned seeds of each species were evenly seeded into the emergence lines or buried in nylon germination bags. Emergence lines were spaced 8 cm apart and constructed along a 1-m strip in each plot. Analogous to the seed treatment in the emergence line, germination bag burials were placed at the same depth, as this allowed for the processes of germination and emergence to be assessed separately. Germination bags and emergence lines were covered with 10 mm of field soil that was sieved at a particle size of 5 mm. Emergence was defined when a seedling coleoptile was visible above the soil surface. The germination bags, which were placed on either end of the emergence lines, were removed from the field 2 weeks (4 January 2013) and 4 weeks (18 January 2013) after the commencement of the trial. After removing remnant soil material from the germination bags, seeds were inspected for germination under a dissection microscope, with germination defined as radicle emergence greater than one-third of the floret or seed coat length.

Quantification of seedbed characteristics

To determine the effects of rainfall on germination and emergence, soil water potentials were calculated across three locations in each site from volumetric soil moisture content (m^3/m^3) measured in the field using HOBO® Micro Station Data Loggers (Onset Computer Corporation, Bourne, MA, USA) fitted with soil moisture probes (EC-5 ECH₂O Dielectric Aquameter; Decagon Devices, Inc.). The probes were buried under approximately 10 mm of sieved field soil (sieved at 5-mm particle size). Soil temperature was also monitored, but only on one location in each site. Measurements were recorded for both moisture content and temperature every 15 min for 4 weeks. The soil temperature range experienced by the seeds under various rainfall conditions was inside the optimal germination conditions for these species (data not shown). To convert volumetric moisture content to soil water potential, water retention curves were determined from soil composite subsamples extracted from 5-mm sieved field soil that reflected the microenvironment of the germination burial bags and seedling emergence lines. Three replicates of at least 5 g were saturated with water to obtain 'field capacity' moisture availability. Samples were then dried back in an oven at 75 °C, with analysis for soil moisture occurring every 10 min with a dew point psychrometer (WP4C Dew Point Potentiometer; Decagon Devices, Inc.). The data obtained from each irrigation site in the field converted to soil water potentials by adjusting the data using the Van Genuchten (1980) model.

STATISTICAL DESIGN

Quantifying germination traits

Time to 50% germination (t_{50}) was calculated across cumulative germination curves for optimal moisture conditions. A three-parameter log-logistic function was used to fit cumulative germination data over time:

$$F(x) = \frac{d}{1 + \exp[b(\log(x) - \log(e))]} \quad \text{eqn 1}$$

To understand the effects of moisture on germination, thresholds were quantified for the untreated florets, wet/dry cycled florets or cleaned seeds by calculating median base water potentials

(Ψ_{b50}). Parameters for Ψ_{b50} for *T. epactia* and *T. wiseana* were calculated by fitting a three-parameter Weibull function across water potential, as Weibull functions are well suited to describe cardinal responses (Ritz 2010):

$$F(x) = 0 + (d - 0) \exp[-\exp(b(\log(x) - \log(e)))] \quad \text{eqn 2}$$

Both nonlinear functions were fitted using the 'drm' function available in the 'drc' package in R (Ritz & Streibig 2005), where (d) is the parameter for maximum germination, (b) the slope of the curve $F(x)$ and (e) the 50% point of the curve ($=t_{50}$; or Ψ_{b50}). Comparisons between calculated parameters were based on approximate t -test of ratios, as described in Ritz & Streibig (2005). For both nonlinear functions, respective functions were chosen against other nonlinear functions, as they provided the best fit based on the log-likelihood and Akaike Information Criterion index.

Field trial germination and emergence responses

Field trial germination and emergence responses were analysed with logistical binomial regressions fitted with a logit-link function and a binomial error structure, using the 'glm' function in R (R Core Team 2014). The full model with interactions was fitted, followed by a stepwise reduction to simplify the final model. The initial model compared germination and emergence between the 2- and 4-week monitoring points to determine whether there was variation in the response as a consequence to natural rainfall. The final model analysed the effects of the main factors of species, seeds at different dormancy states, rainfall frequency and rainfall quantity and their interactions across the rainfall scenarios using the 2 week monitoring data point.

Seedbed characteristics and modelled cumulative time above Ψ_{b50} for untreated or wet/dry cycled florets, and cleaned seeds

For each week, mean soil water potentials and temperatures were compared using repeated-measures ANOVAS between rainfall sites, and the overall variation between rainfall combinations determined through Tukey's Honestly Significant Difference (HSD). Cumulative mean time soil water potentials above base water potentials ($\Psi_{\text{soil}} \geq \Psi_{b50}$) were then calculated by the sum of hours when $\Psi_{\text{soil}} \geq \Psi_{b50}$ for seeds in different dormancy states separately within rainfall sites. Final cumulative time $\Psi_{\text{soil}} \geq \Psi_{b50}$ was compared using a two-way ANOVA on rainfall combination and germination unit to quantify final time accumulation and Tukey's HSD to determine differences within rainfall sites after 2 weeks following initiation of the trial, as any time point after did not accumulate additional time for the remaining time of the field trial.

Results

GERMINATION OF SEEDS TREATED TO EXHIBIT DIFFERENT DORMANCY STATES UNDER OPTIMAL CONDITIONS AND WATER STRESS

Germination of wet/dry cycled florets and cleaned seeds under non-limiting laboratory conditions demonstrated

Fig. 1. Germination dynamics for *Triodia epactia* and *Triodia wiseana* in optimal (top panels) and water stress (bottom panels) conditions fitted for raw germination responses (not cut test adjusted) of untreated florets, wet/dry cycled florets (-WD) and cleaned seeds. Time to 50% germination (T_{50}) coefficients was estimated by fitting three-parameter log-logistic functions onto final germination responses for optimal water conditions ($\Psi = 0$ MPa). Base water potentials (Ψ_{b50}) were modelled by fitting three-parameter Weibull functions onto normalised germination responses (germination upper limit equal to 1) across a water potential gradient created by different polyethylene glycol solutions. Lower case letters are significant differences at $P < 0.05$. Full model parameter outputs are supplied in Appendix S1.

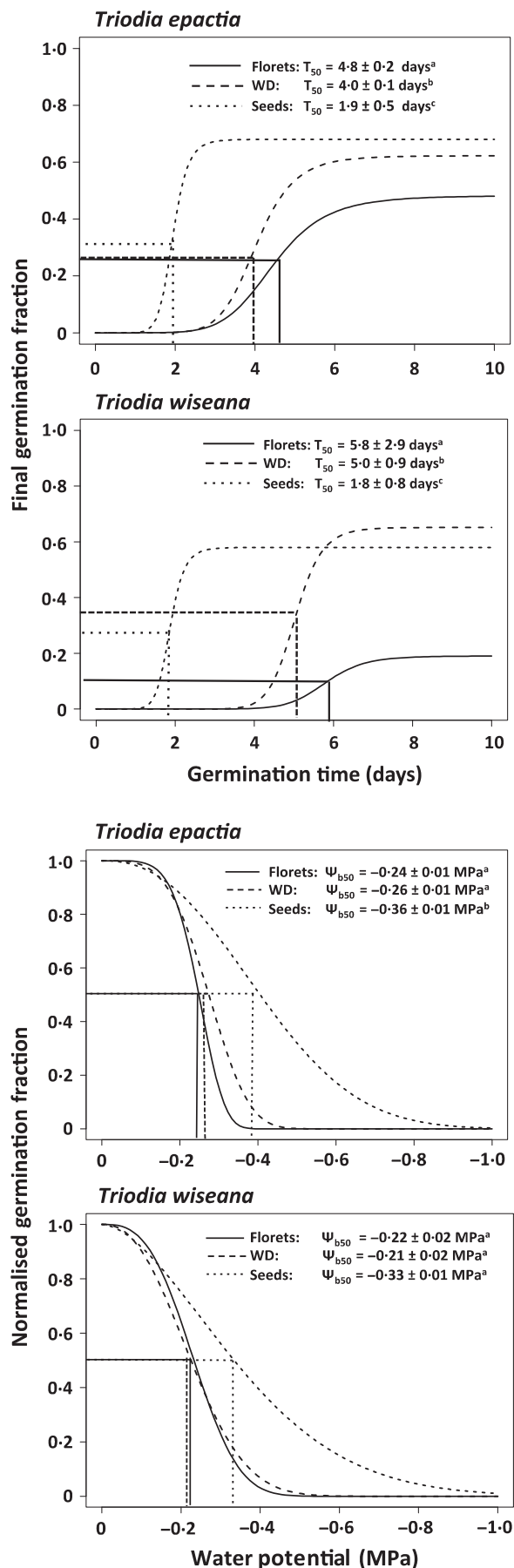
significantly greater germination (up to 68%) when compared to untreated florets (<50%) in both species (all $P < 0.001$; Fig. 1). Furthermore, treating seeds by wet/dry cycling florets or cleaning to seed resulted in faster germination (lower t_{50}), with untreated florets consistently germinating slowest (all $P < 0.0001$; Fig. 1). Cleaned seeds, however, germinated at least twofold and 2.5-fold faster than wet/dry cycled florets in *T. epactia* and *T. wiseana*, respectively (Fig. 1).

Modelled Ψ_{b50} thresholds were the lowest from intact florets in *T. epactia* ($\Psi_{b50} = -0.24$ MPa) and similarly in *T. wiseana* ($\Psi_{b50} = -0.22$ MPa), indicating that intact florets were more sensitive to water stress than cleaned seeds in both species (Fig. 1). Although cleaned seeds demonstrated the lowest Ψ_{b50} thresholds in both species, *T. epactia* showed a slightly more negative threshold than *T. wiseana* (Fig. 1).

INTERACTIONS OF RAINFALL DYNAMICS ON GERMINATION AND EMERGENCE

In the field, germination (<35%) and emergence (<10%) were limited to watered sites during the first 2 weeks only, with no new germination or emergence observed at the 4-week monitoring point. In cleaned seeds, the treatment resulting in the most negative Ψ_{b50} , watering contributed to significant variation in the germination response across the different simulated rainfall combinations, but seedling emergence was limited to frequent events totalling 48 and 96 mm (Fig. 2).

Maximum germination was observed from the 4×24 mm rainfall treatment, with germination between 28% and 35% and seedling emergence between 8 and 10% (Fig. 2). These responses were on average twofold higher than from the 4×12 mm event ($P < 0.0001$; Fig. 2). Increasing the frequency, regardless of the quantity, significantly improved germination and emergence for both species ($P < 0.0001$; Fig. 2). Furthermore, from the 4×24 mm event when compared to the 2×48 and 1×96 mm events, or from 4×12 mm event when compared to 2×24 and 1×48 mm events, germination increased by up to fourfold ($P < 0.0001$; Fig. 2). Seedling emergence was completely absent in single frequency events in both species (Fig. 2).



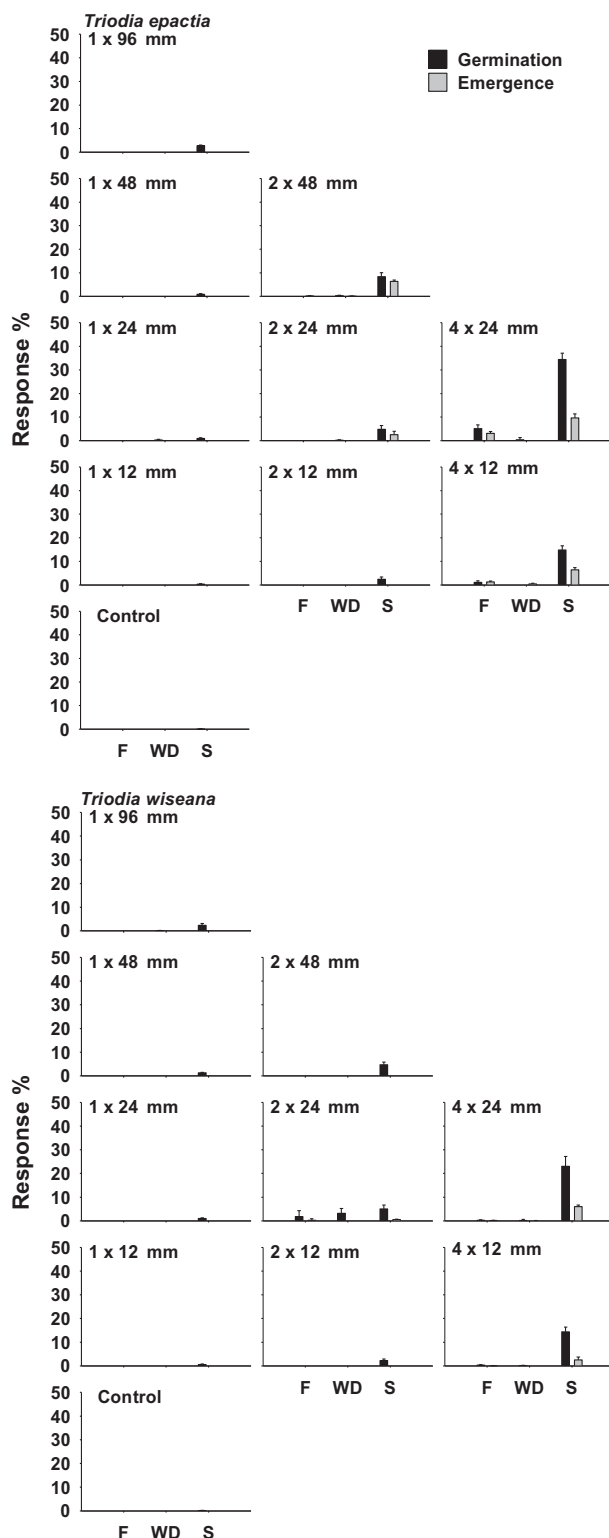


Fig. 2. Effects of rainfall frequency and quantity on mean germination (black bars) and emergence (grey bars) from the total number of pre-treated florets and seeds of *Triodia epactia* and *Triodia wiseana* sown per rainfall combination. Germination and emergence data are shown as the raw data (not cut test adjusted), with untreated florets were the control for both species, while dormancy states were florets that were wet/dry cycled (-WD) or cleaned seeds. Error bars are one standard error of the mean, $n = 5$.

Although *Triodia* species were showing similar patterns to the different simulated rainfall events, differences existed between species with germination and emergence responses significantly higher in *T. epactia* ($P < 0.05$; Fig. 2). While observed in the 2× or 4× frequency events in *T. epactia*, seedling emergence was confined to 4× frequency event in *T. wiseana* (Fig. 2).

Seedbed characteristics and modelled cumulative time above Ψ_{b50} for untreated or wet/dry cycled florets, and cleaned seeds

Occasional rainfall totalling 2 mm occurred during week 1 of the trial period, but was negligible in terms of the much larger amounts applied as artificial watering treatments (Fig. 3). Overall Ψ_{soil} varied the most during week 1, in response to the simulated rainfall treatments (Fig. 3; Table 1), whereas in weeks 2, 3 and 4 soil characteristics were more similar, but became drier and hotter throughout the experiment (Table 1). The total delivery quantity was significant for increasing Ψ_{soil} , with higher quantity thresholds achieved by frequent rainfall increasing Ψ_{soil} ranges (Table 1). For example, frequent rainfall events that delivered 48 and 96 mm in total showed higher Ψ_{soil} between (−0.22 to −0.24 MPa), compared to 24 mm (−0.27 to −0.31 MPa).

To relate calculated germination thresholds to Ψ_{soil} from different rainfall scenarios, cumulative time of Ψ_{soil} above the Ψ_{b50} was quantified. This was assessed across different simulated rainfall scenarios with a total rainfall of 48 or 96 mm (Fig. 4). As cleaned seeds were showing more negative Ψ_{b50} than any of the intact floret treatments, Ψ_{soil} was held for a significantly longer time (all $P < 0.001$) within the simulated rainfall treatments compared to intact floret treatments (Fig. 4). The lowest cumulative times for $\Psi_{soil} \geq \Psi_{b50}$ occurred in ambient rainfall conditions (<90 h), while 2× and 4× frequency rainfall events enabled cumulative time above 160 h. From the rainfall events (i.e. 4 × 24 mm, 4 × 12 mm or 2 × 48 mm) that produced highest germination and emergence responses for cleaned seeds cumulative times were 168–213 h, approximately 1.6- to 2.6-fold higher cumulative time than intact florets (65–130 h) in both species.

Discussion

This study is the first to quantify and integrate the impacts that seed dormancy, and its alleviation, has on recruitment success within various climatic conditions under simulated field conditions. Unique to our study, we have shown that seed dormancy is a major cause of recruitment failure, by delaying germination and emergence rates after large frequent rainfall events in the two grass study species, *T. epactia* and *T. wiseana*. By creating three dormancy states through seed pre-treatment manipulations: highly dormant (florets; 19–49% germination, narrow germination window $\Psi_{b50} = -0.22$ to -0.24 MPa), moderately dormant

(wet dry florets; 62–65% germination, moderate germination window $\Psi_{b50} = -0.21$ to -0.26 MPa), least dormant (cleaned seed; 58–68% germination, wide germination window $\Psi_{b50} = -0.33$ to -0.36 MPa) and exposing them to large frequent rainfall events (e.g. 4×24 mm), we tested the hypothesis that seed dormancy limits germination and emergence under field conditions. This hypothesis was accepted with the least dormant state of cleaned seed achieving c. 35% germination and 10% emergence, over fivefold more than that achieved using other dormancy alleviating treatments. Moderately dormant propagules, despite improving final germination performance under optimal water conditions (Fig. 1), failed to increase germination and emergence above the most dormant state (over 90% of germination failure) under field conditions. While improving the germination envelope by creating less dormant seed propagules demonstrates the capacity to promote recruitment success under the best performing rainfall scenario (4×24 mm; $\Psi_{soil} = -0.22$ MPa), an important finding is that recruitment potential, even from non-dormant seeds, is innately limited by rainfall.

For dormant seeds, failure to capitalise in short time frames on favourable moisture conditions may compromise recruitment outcomes further. Seed dormancy has been proposed to provide an ecological advantage through bet hedging [protecting seeds from germinating into suboptimal establishment conditions (Philippi 1993; Fenner & Thompson 2005)]. For example, grass florets in the arid zone may persist for up to 2 years in the seed bank (Pyke 1990; Simpson 1990) providing several recruitment opportunities. Although bet hedging strategies were not explored here, given the short time frame of observations (4 weeks), the decline in seed viability of up to 40% in the field (see Appendix S2), which was independent of initial dormancy state, may promote the use of seed dormancy alleviation practices to capitalise on recruitment opportunities before seed bank decline occurs. Understanding longer-term seed persistence after manipulating dormancy will be critical to understand impacts on the role of dormancy in facilitating recruitment strategies across global arid systems and to inform restoration seed mixes.

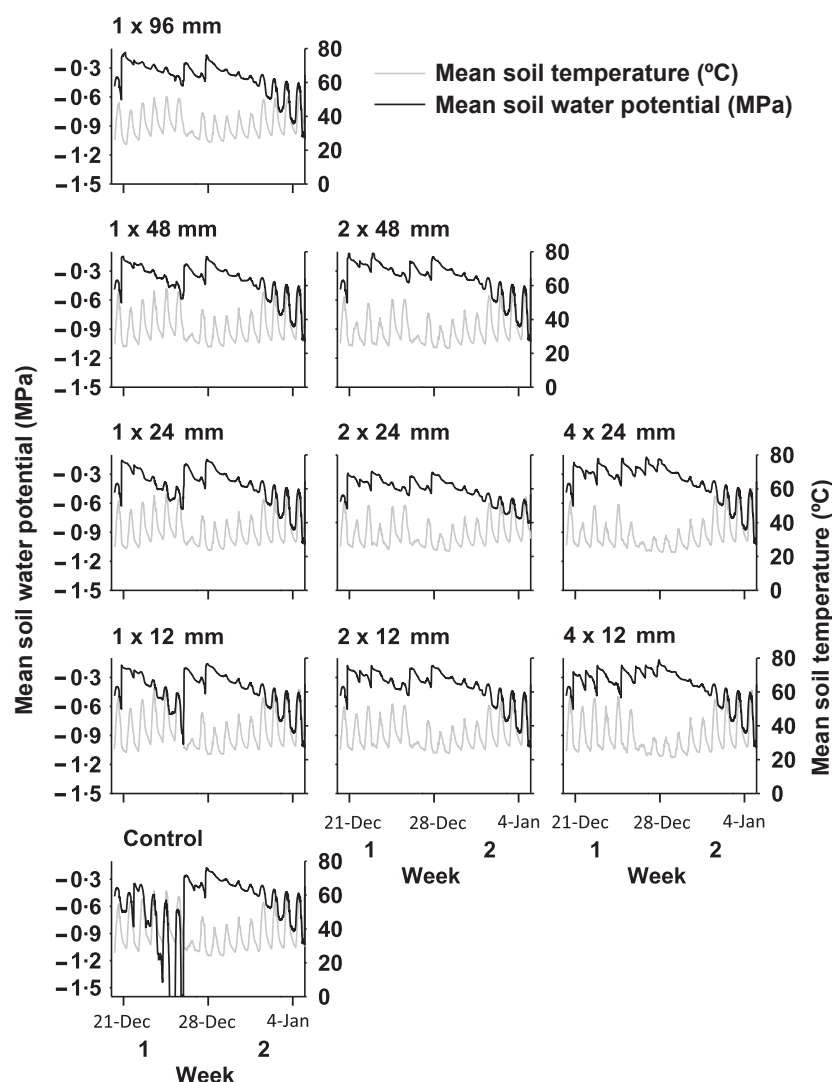


Fig. 3. Mean soil water potential (MPa) and mean soil temperature ($^{\circ}\text{C}$) characteristics in experimental rainfall sites across 2 weeks. The rainfall delivery period was conducted during week 1, with occasional natural rainfall (totalling 2 mm) occurring in the control and across artificially watered sites during this period. All blocks were exposed to the same conditions from week 2 to 4. Soil moisture was measured by converting volumetric moisture content into soil water potentials using the Van Genuchten (1980) model.

Table 1. Mean soil water potential (Ψ_{soil}) and mean soil temperature (T_{soil}) (\pm standard error) for each week during the trial period. Letters indicate significant differences at $P < 0.05$ between rainfall combinations within week periods. Artificial watering occurred in week 1. Natural rainfall of 2 mm during week 1 contributed to elevated Ψ_{soil} in the control site and additionally to artificial watering treatments

Rainfall scenario		Week 1 (Delivery of Event)		Week 2		Week 3		Week 4	
Total quantity threshold	Rainfall frequency \times Rainfall quantity	Ψ_{soil} (MPa)	T_{soil} ($^{\circ}\text{C}$)	Ψ_{soil} (MPa)	T_{soil} ($^{\circ}\text{C}$)	Ψ_{soil} (MPa)	T_{soil} ($^{\circ}\text{C}$)	Ψ_{soil} (MPa)	T_{soil} ($^{\circ}\text{C}$)
Control	Ambient	-0.66 ± 0.03^a	38.0 ± 0.3^a	-0.43 ± 0.13^a	36.9 ± 0.7^a	-0.63 ± 0.02^a	40.3 ± 0.7^a	-1.10 ± 0.04^a	39.4 ± 0.7^a
12 mm	1 \times 12 mm	-0.34 ± 0.01^b	36.9 ± 0.4^b	-0.42 ± 0.01^a	36.7 ± 0.8^a	-0.60 ± 0.02^a	40.2 ± 0.8^a	-0.99 ± 0.01^a	39.0 ± 0.8^a
24 mm	1 \times 24 mm	-0.31 ± 0.01^b	35.4 ± 0.3^c	-0.43 ± 0.01^a	35.9 ± 0.8^a	-0.58 ± 0.03^a	39.4 ± 0.6^a	-0.99 ± 0.02^a	39.7 ± 0.6^a
48 mm	2 \times 12 mm	-0.27 ± 0.01^c	33.9 ± 0.3^d	-0.43 ± 0.01^a	35.4 ± 0.6^b	-0.59 ± 0.02^a	39.0 ± 0.6^a	-0.97 ± 0.02^a	38.8 ± 0.6^a
	1 \times 48 mm	-0.29 ± 0.01^b	35.7 ± 0.3^c	-0.42 ± 0.02^a	36.5 ± 0.7^a	-0.63 ± 0.01^a	39.4 ± 0.7^a	-1.00 ± 0.02^a	39.0 ± 0.7^a
	2 \times 24 mm	-0.24 ± 0.01^d	32.1 ± 0.3^e	-0.42 ± 0.03^a	35.0 ± 0.8^b	-0.58 ± 0.03^a	39.7 ± 0.6^a	-0.95 ± 0.02^b	38.9 ± 0.6^a
96 mm	4 \times 12 mm	-0.23 ± 0.02^d	32.1 ± 0.3^c	-0.40 ± 0.02^a	35.0 ± 0.9^b	-0.58 ± 0.02^b	39.5 ± 0.8^a	-0.99 ± 0.01^a	39.3 ± 0.8^a
	1 \times 96 mm	$-0.28 \pm 0.02^{b,c}$	35.0 ± 0.2^f	-0.39 ± 0.04^a	35.6 ± 0.5^b	-0.59 ± 0.01^a	39.0 ± 0.9^a	-1.10 ± 0.02^a	38.6 ± 0.6^a
	2 \times 48 mm	$-0.23 \pm 0.02^{d,c}$	32.6 ± 0.2^g	-0.42 ± 0.01^a	35.7 ± 0.7^a	-0.57 ± 0.03^b	39.5 ± 0.7^a	-0.98 ± 0.01^a	38.7 ± 0.7^a
	4 \times 24 mm	-0.22 ± 0.01^c	30.3 ± 0.2^h	-0.41 ± 0.01^a	35.2 ± 0.8^b	-0.59 ± 0.02^a	39.0 ± 0.6^a	-1.00 ± 0.01^a	38.8 ± 0.6^a

Although it has previously been established that a relatively small proportion of seeds will establish from sown seeds (O'Connor 1996; Chambers 2000; James, Svejcar & Rinella 2011), this study provides the mechanistic basis of this limitation, indicating a multiphasic recruitment bottleneck during establishment between seed (30–35% germination) and seedling (48–50% emerged from the germination fraction) even under an imposed 95th percentile rainfall conditions (4 \times 24 mm, Fig. 2; Table 1). Indeed, our results show that seed dormancy state alters the overall soil moisture threshold ($\Psi_{\text{soil}} \geq \Psi_{\text{b50}}$) that is required to drive germination and emergence. We suggest that the term 'establishment' used should therefore be documented clearly in two phases: (i) germination and (ii) emergence and abiotic/biotic interactions with both should be considered to inform demographic recruitment processes.

A fundamental gap in current knowledge is the rainfall requirements of *Triodia* to initiate germination and emergence in the field at the finer scale of seedling recruitment. Our hypothesis was that the frequency of rainfall events is significant for triggering recruitment events, with only those events maintaining base water potentials (Ψ_{b50}) successfully driving germination and emergence. This was supported, however, even under our best performing seed pre-treatment, seedling emergence was limited to 10% under the highest rainfall frequency and rainfall quantity combination (i.e. 4 \times 24 mm). Any decrease in the rainfall frequency and quantity reduced seedling germination and emergence to <5% and 2.5%, respectively. Seed dormancy state further inhibited recruitment processes with dormant florets having a narrow moisture threshold (requiring more frequent and greater soil moisture) (Fig. 1), characteristic of conditionally dormant species (Meyer, Allen & Beckstead 1997; Baskin & Baskin 2014) and possibly the result of a reduced germination rate (Mott 1974; Gallart, Verdú & Mas 2008). Furthermore, the overall finding that seedling emergence is triggered by frequent

rainfall generally agrees with other studies (Yang *et al.* 2010; Zhu *et al.* 2014), although these studies do not associate emergence processes with available moisture such as critical thresholds (e.g. Ψ_{b50} , as used in this study). Increasing the germination envelope through dormancy alleviation provides a pathway for improving germination and emergence success, and further emphasis should be placed on seed/soil interactions to promote recruitment processes.

The findings from this study strongly suggest that seedling recruitment in arid ecosystems such as the Pilbara is favoured by a period of continuous rainfall at which recruitment is successfully triggered from larger cyclonic rainfall events. Of ecological importance are the two key size-class models characterising scale and distribution of rainfall (Noy-Meir 1973; Schwinning & Sala 2004). Large rainfall pulses, such as the 24–96 mm quantities, occur over a longer duration between 2 and 3 days through frequent rainfall events (Dare, Davidson & McBride 2012) while smaller single rainfall events <5 mm more frequently occur as disjoint pulses and the duration between pulses is >10 days (Noy-Meir 1973; Schwinning & Sala 2004). These larger, continuous events in the Pilbara region are more likely to occur during La Niña rather than El Niño periods (Liu & Chan 2012). Predictive modelling of Pilbara rainfall suggests an overall increase in the number of low pressure rainfall days with a decrease in cyclone frequency (Charles *et al.* 2013; Lavender & Abbs 2013). The increasing low pressure rainfall days, often associated with <5 mm rainfall events, may not provide sufficient moisture to trigger germination and support emergence, unless these events are continuous, which is twice as likely under La Niña rather than El Niño periods (Liu & Chan 2012). The variability of recruitment in *Triodia* in this study therefore reflects climatic variation on larger scales, with recruitment of arid-zone species (e.g. O'Connor 1996; Bowers, Turner & Burgess 2004; Muñoz-Rojas *et al.* 2016) linked to above average rainfall years.

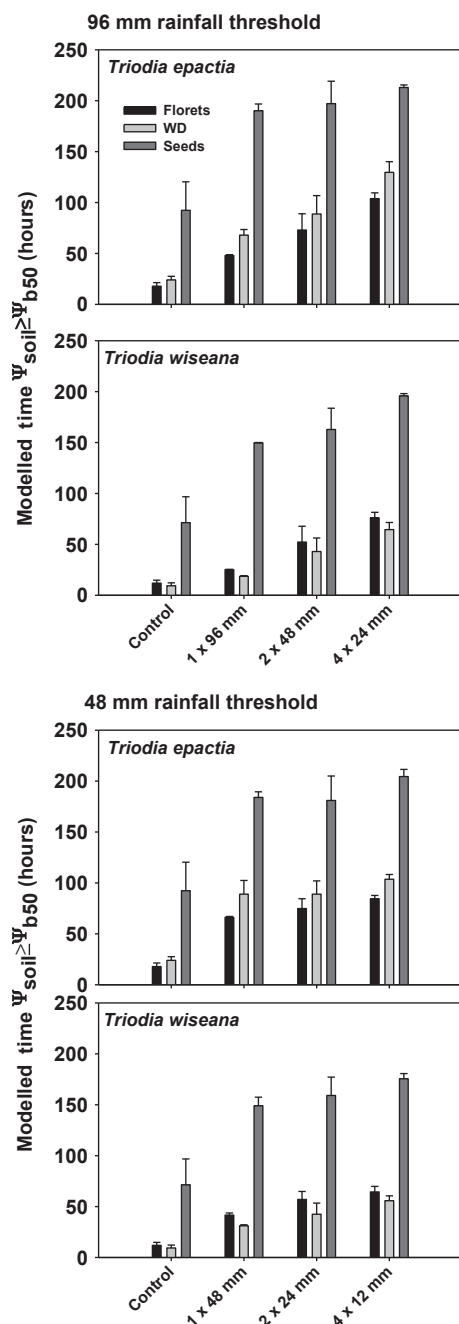


Fig. 4. Modelled cumulative time $\Psi_{\text{soil}} \geq \Psi_{b50}$ across artificially watered sites for (untreated) florets, wet/dry cycled florets (-WD) and cleaned seeds. Natural rainfall of 2 mm during week 1 contributed to elevated Ψ_{soil} in the control site and additionally to artificial watering treatments. Error bars are one standard error of mean, $n = 3$.

With arid ecosystems having high vapour pressure deficit coincident with many soils having low water retention capacity (Fernandez-Illescas *et al.* 2001), soil drying rates will dictate time for $\Psi_{\text{soil}} \geq \Psi_{b50}$. Increasing soil water retention capacity in post-mined restoration sites can be achieved through (i) organic amendments, although effects are variable and dependent on the level of aridity (e.g. Bainbridge *et al.* 2001; Benigno, Dixon & Stevens 2012);

(ii) deep ripping (Knight, Beale & Dalton 1998; Rokich *et al.* 2001), or soil raking (Turner *et al.* 2006) that create germination/emergence microsites by establishing niches in cracks or under rocks to focus water on the seed zone (Harper, Williams & Sagar 1965); (iii) manipulation of soil texture to improve soil water retention (Chambers 2000); or (iv) supplementing restoration sites with artificial irrigation particularly during dry years (e.g. Bainbridge *et al.* 2001). Alternatively, treatments to the seed through priming or coating technologies should aim at improving germination vigour (Erickson *et al.* 2016; Madsen *et al.* 2016) while enabling seed through use of stress tolerance agents such as salicylic acid (Stevens, Senaratna & Sivathamparam 2006) to improve stress tolerance.

Despite *T. epactia* and *T. wiseana* populations co-occurring in the arid landscape of Australia, *T. wiseana* populations occur more frequently in the more arid interior compared to *T. epactia* (Lazarides 1997). Therefore, we expected that this drought tolerance may be the result of seed traits conferring drought adaptation (germination under lower moisture availability). This was not the case, with *T. epactia* able to germinate and emerge from drier conditions than *T. wiseana* in the field (2 x 48 mm events; Fig. 2), and both having similar germination traits under controlled water stress conditions (*T. epactia* a -0.36 MPa; *T. wiseana* -0.33 MPa). With seed dormancy associated with being a drought avoidance strategy, it is expected that this trait will be reflected across different *Triodia* populations, within and between species to varying degrees. Future research should therefore test multiple seed accessions from different populations across a wide aridity gradient to fully assess species level differences (e.g. see germination variation of two *T. wiseana* accessions in Erickson *et al.* 2016).

While other studies have shown over 80% of seedling mortality occurring during the first season of drought after emergence (Pyke 1990; Chambers 2000), seedling survival in this study after 1 year was observed to be approximately 30% in both species (data not shown). This loss may be conservative given the above average rainfall year experienced (BOM 2016); however, understanding impacts of longer-term rainfall patterns on plant establishment in disturbed and undisturbed systems is required.

Conclusion

This study highlights that a major impediment in restoration occurs at the germination–emergence phases, and both phases should be considered independently. Seed dormancy and variable rainfall were found to be the primary obstacle to blocking recruitment processes for both *Triodia* species therefore highlighting benefits for alleviating seed dormancy prior to seeding in restoration sites, as this increases the envelope for germination. Large frequent rainfall events (e.g. 4 x 24 mm) were found to be critical for raising soil water potentials to support

recruitment processes for seeds at different dormancy states, but highest germination and emergence responses observed with cleaned seeds that were considered the least dormant with the widest germination envelope for water stress. By relating the germination envelope of seeds at different dormancy states to soil water potentials after rainfall, this concept will guide arid-zone restoration practices including seed treatments, substrate design, restoration timing (e.g. La Niña years) and irrigation intervention.

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Data accessibility

All data in this manuscript are archived in Dryad Digital Repository <http://dx.doi.org/10.5061/dryad.br8s9> (Lewandrowski et al. 2016).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Viability state and curve coefficients from nonlinear functions in different dormancy states after receiving treatment.

Appendix S2. Final viability of intact florets and cleaned seeds after exposing to different simulated rainfall events in the field.