Seed and seedling traits affecting critical life stage transitions and recruitment outcomes in dryland grasses

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Summary

1. Seeding native plants is a key management practice to counter land degradation across the globe, yet the majority of seeding efforts fail, limiting our ability to accelerate ecosystem recovery.

2. Recruitment requires transitions through several seed and seedling stages, some of which may have overriding influences on restoration outcomes. We lack, however, a general framework to understand and predict differences in these critical demographic processes across species. Functional traits influence fitness, and consequently, trait variation could provide the basis for a framework to explain and predict variation in life stage transition probabilities.

3. We used seed and seedling traits, and field probabilities of germination, emergence, seedling establishment, and survival for 47 varieties of drylands grasses under two watering treatments to identify critical life stage transitions and quantify the effect of traits on cumulative survival through the first growing season.

4. Variation in germination and emergence probabilities explained over 90% of the variation in cumulative survival regardless of seedling survival probabilities or watering treatment, with emergence probability being the strongest predictor of cumulative survival.

5. Coleoptile tissue density and seed mass had significant effects on emergence and germination, respectively, explaining 10–23% of the variation in transition probabilities.

6. *Synthesis and applications.* While the majority of functional trait work has centred on linking leaf and root traits to resource acquisition and utilization, our study demonstrates that traits associated with germination and emergence may have prevailing influences on restoration outcomes. A portion of these traits have been examined, but there is substantial opportunity to identify other key traits driving these demographic processes. These advancements will underpin our ability to develop trait-based frameworks for overcoming recruitment barriers and facilitating recovery of degraded systems across the globe.

Key-words: coleoptile tissue density, demography, emergence, functional traits, germination, hydrothermal time, path analysis, seed mass, seeding

Introduction

Land degradation driven by altered disturbance regimes and environmental change is one of the largest threats to terrestrial ecosystem structure and function across the

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globe (Millennium Ecosystem Assessment 2005; Bai *et al.* 2008). In many systems, without active intervention, plant communities remain in degraded states with a reduced capacity to supply critical ecosystem services (Levine *et al.* 2003; Seabloom *et al.* 2003). Seeding native species is a common management tool to restore ecosystem function, but costs are high and efforts routinely fail to meet restoration goals (Doust, Erskine & Lamb 2008; Abella *et al.* 2009; Pyke, Wirth & Beyers 2013). If seeding is going to be a useful tool to restore degraded land, we must drastically enhance our understanding of the ecological processes and mechanisms driving recruitment outcomes.

Recruitment is composed of several key demographic processes, some of which could have disproportionate influences on recruitment outcomes. Key life stage transitions include germination, the transition from a quiescent or dormant seed to a germinated seed; emergence, the transition from a germinated seed to an emerged seedling; establishment, the transition from a newly emerged seedling to an autotrophic seedling; and seedling survival, the persistence of established seedlings through the first growing season (Fenner & Thompson 2005). While it is well understood that the bulk of population mortality often occurs during recruitment (e.g. Zeiter, Stampfli & Newbery 2006; Gómez-Aparicio 2008), we have a fragmented understanding of which transitions drive these large mortality events, and thus, how management strategies can be refined to mitigate mortality. Research to date suggests that germination and emergence can be major constraints (e.g. Pyke 1990; Ryser 1993; Rebollo *et al.* 2001; Clarke & Davison 2004), though the relative contribution of these transitions to recruitment is rarely compared. Efforts to partition these relative effects focus on only a handful of species (Hegarty & Royle 1978; James, Svejcar & Rinella 2011), limiting our understanding of the broader demographic processes that drive relative abundances in managed systems. Quantifying the relative influence of life stage transitions on recruitment variation across species and environmental conditions is a critical first step towards isolating underlying physiological and morphological traits that influence recruitment outcomes.

Plant functional traits provide a fundamental unit of comparison to explain and predict differences in performance among species within a common environment (McGill *et al.* 2006). Cornwell & Ackerly (2010) found functional traits such as specific leaf area (SLA) and height to be predictors of local abundance in coastal California communities, while Martínez-Garza, Bongers & Poorter (2013) linked leaf and canopy traits to survival probabilities among co-occurring tropical tree species. While progress to date has been valuable, plant traits have largely been linked to community assembly and established plant distributions along environmental gradients with less understanding of how trait variation influences underlying demographic processes – particularly during key recruitment stages. Tying variation in seed and seedling demographic rates to functional trait variation

could provide a general trait-based framework to predict recruitment outcomes, guide selection of restoration species and to identify and manage key environmental filters mitigating trait–recruitment relationships.

Direct links between traits and demographic transitions have yet to be examined across multiple stages of recruitment. Traits associated with seedling performance are most studied, when the ability to procure and conserve resources may influence survival. On one end of a tissue economics trait spectrum, species constructing less dense leaf or roots create more absorptive surface area per unit biomass allowing rapid resource acquisition and growth. On the other end, dense leaf and root tissues promote resource conservation and may enable survival during resource-limited periods (Wright & Westoby 1999; Reich *et al.* 2003). Comparatively less is known about traits driving germination and emergence. Among traits influencing germination, seed mass is the most investigated and is typically positively associated with germination across a range of environments (Pearson *et al.* 2002; Kahmen & Poschlod 2008). Hydrothermal time quantifies the accumulation of temperature and moisture required for germination and could serve as a physiological index of germination rate and timing (Allen, Meyer & Khan 2000). To date, however, hydrothermal time concepts have not been integrated into trait frameworks. Finally, seed mass and coleoptile tissue density reflect the amount of reserves available to the coleoptile and how they are invested, respectively. They may have positive effects on emergence by conferring greater physical support to penetrate the soil surface (Andrews *et al.* 1997; Gardarin, Dürr & Colbach 2010) or reducing the susceptibility of germinated seeds to fungal attack and drought, respectively (Crist & Friese 1993; Ganade & Westoby 1999).

Relationships between traits and demographic processes may be complex given the role of multiple abiotic and biotic factors in determining the degree of selection on traits (Keddy 1992). Recruitment depends on early demographic processes, which may be especially sensitive to variability in precipitation, temperature or other conditions. High-precipitation years could result in uniformly high recruitment rates regardless of trait variation (e.g. Engelbrecht, Kursar & Tyree 2005); however, other studies have found trait–recruitment relationships to be unaltered by substantial water addition (Leishman & Westoby 1994). It is thus integral to understand how demographic processes and trait influence vary as a function of environmental gradients.

The objective of this study was to test a trait-based framework for recruitment across drylands grasses by identifying the critical demographic processes driving recruitment probability, examining the ability of functional traits to predict these processes and assessing how water availability alters these relationships. Using path analysis, we quantified links between traits, life stage transition probabilities, and first-year cumulative survival under two watering scenarios to test the following

hypotheses: (i) Germination and emergence probabilities are the most critical transitions driving variation in cumulative survival probabilities during the first growing season, and accordingly, early functional traits including seed mass, hydrothermal time and coleoptile tissue density will be key predictors of recruitment success. (ii) Higher spring soil moisture will increase germination and emergence probabilities across grasses and therefore reduce the degree to which variation in these functional traits influence recruitment.

Materials and methods

STUDY SYSTEM AND EXPERIMENTAL APPROACH

Trait and life stage transition data were collected at the Northern Great Basin Experimental Range near Burns, Oregon, US (1400 m elevation, 283 mm average annual precipitation). Data were collected for 47 species and varieties (i.e. ecotypes or cultivars) of grasses broadly distributed across dryland systems of western North America, including key native and non-native perennials used in restoration and four introduced annuals (see Table S1 in Supporting Information). We arranged 300 tilled 1-m^2 plots in a 15 × 20 grid and randomly assigned each variety to six plots. Plots were hand-sown (400 seeds plot^{-1}) to monitor life stage transitions. Plots also contained one germination bag (50 seeds bag^{-1}) and two open-top seedling bags made of nylon mesh, filled with field soil and buried at depths of 2–5 cm and 0–10 cm, respectively, to quantify germination probabilities and seedling traits. The experimental design is shown in Fig. S1 (Supporting information).

Seeds were sown in November 2011. Precipitation for the study year (130 mm) was <50% of average; therefore, all plots were supplemented with 65 mm of water over six April watering events, prior to emergence, and row coverings (Agribon AG-19) were temporarily placed over plots during watering to reduce evaporative loss. To examine the influence of water availability on traits and transition rates, half of the plots per variety received an additional 65 mm watering treatment distributed as 12 events (ranging 2·5–10 mm) between March and May 2012, allowing us to examine a relatively average water year and a wetter water year scenario.

LIFE STAGE TRANSITION PROBABILITIES

To account for differences in seedlot viability, tetrazolium (TZ) testing was performed when prior viability tests were unavailable or indicated <90% germinability (Appendix S1, Supporting information). TZ testing followed the Association of Official Seed Analysts and was conducted at the Oregon State University Seed Laboratory (Corvallis, OR, USA). Conditional life stage transition probabilities (Table 1; Fenner & Thompson 2005) were calculated from three germination bags and seeded plots per grass variety and treatment. Germination probability was the average proportion of germinated seeds (radical > 2 mm) in germination bags multiplied by seedlot viability. Emergence and seedling mortality were monitored in plots biweekly from April to dormancy onset in late June. Emergence was the average proportion of germinated seeds (germination probability · 400 sown seeds plot^{-1}) with coleoptiles penetrating the surface. Establishment was the average proportion of emerged seedlings surviving to establishment, defined as the transition to autotrophism and indicated by full expansion of the first true leaf. Established seedling survival

Table 1. Collected trait and life stage transition data. All variables were measured under two watering treatments except seed mass, coleoptile tissues density and hydrothermal time. Seedling traits were measured at two harvests: 'early' traits at seedling establishment and 'late' traits 3 weeks later. Seedling trait transformations were performed within harvest and treatment (e_w = early watered, l_w = late watered, l_{nw} = late non-watered)

Variables	Definition	Original Units	Transformation
Life stage transitions			
	Per germination bag		
Germination	No. germinated seeds/(Total no. seeds · % Viability*)	%	Logit
	Per plot		
Emergence	No. emerged seedlings/(Total seeds sown · % Germination*)	%	Logit
Seedling establishment	No. established seedlings/No. emerged seedlings	%	Logit
Seedling survival	No. surviving established seedlings/No. established seedlings	%	Logit
Cumulative survival	Total survived seedlings/(Total seeds sown · % Viability*)	%	Logit
Traits			
Seed mass	Dry mass per seed	mg	
Coleoptile tissue density (CTD)	Dry coleoptile mass/Fresh mass	g·g^{-1}	
Hydrothermal time	Required accumulation of temperature and water potential above base levels for germination to occur (based on germination rates up to 25%)	MPa °C·d	Log
Seedling traits in PCA			
Leaf dry matter content (LDMC)	Dry leaf mass/Fresh mass	g·g^{-1}	Log (e_w, l_{nw})
Specific leaf area (SLA)	Leaf area/Dry leaf mass	cm^2 g^{-1}	
Root mass (RM)	Total dry root mass	mg	Log (e_w, l_{nw})
Root length (RL)	Total root length	cm	
Specific root length (SRL)	Root length/Dry root mass	cm·g^{-1}	Log (e_w, l_w)
Root mass ratio (RMR)	Dry root mass/Dry total mass	g·g^{-1}	

*Mean value for variety.

was the proportion of established seedlings surviving to dormancy onset. Finally, cumulative survival was the proportion of sown seeds resulting in survived seedlings. Demographic data are provided in Appendix S1 (Supporting information).

TRAIT SAMPLING

With a limited sample size (*n* = 47 grasses), we utilized the literature to identify key traits with the greatest potential to influence transition probabilities. While leaf and root traits are more frequently examined, traits of seeds and early seedlings are less commonly assessed (e.g. one or two seed traits within the international TRY data base (Kattge *et al.* 2011) and trait handbooks (e.g. Pérez-Harguindeguy *et al.* 2013). To extend this base, we drew upon the crop science literature to identify two additional traits with functional implications, hydrothermal time and coleoptile tissue density. Trait data are summarized in Table 1 and provided in Appendix S1 (Supporting information).

Seedling traits were sampled in spring 2012 from three seedlings per variety, treatment and harvest date. Once seedlings in all open-top bags of a variety and treatment had established, seedlings were thinned to one seedling of uniform size per bag. One set of open-top bags was harvested immediately ('early' traits), and a second set harvested 3 weeks later ('late' traits). Fresh leaves and roots of seedlings were scanned (WinRHIZO, Regent Instruments Inc., Sainte-Foy, Canada) for area and length, respectively, and leaves were kept moist to collect leaf fresh mass within 24 h. Roots, leaves and shoots were dried at 60 °C for 3 days and weighed.

Coleoptile tissue density (CTD) was calculated as the ratio of dry to fresh mass of newly emerged coleoptiles (<1 cm tall) grown in five regularly watered pots per variety outside of the Eastern Oregon Agricultural Research Center greenhouse in Burns, Oregon, in spring 2012 (Ganade & Westoby 1999). Seed mass was the average seed weight from three lots of 50 seeds, oven-dried for 2 days at 60 °C.

To incorporate differences in germination physiology, we sought an index capable of capturing germination response to moisture and temperature. Hydrothermal time is a labour intensive though robust metric, with higher values requiring a greater accumulation of moisture and temperature above base levels to germinate (Gummerson 1986). To estimate hydrothermal time, germination trials were carried out in a factorial experiment with three temperatures (6, 12 and 18 °C) and three water potentials (−0·033, −0·5 and −1·0 MPa), with six replicates per grass variety and treatment. Experimental methods and generation of cumulative germination curves closely followed Hardegree, Flerchinger & Van Vactor (2003). Hydrothermal time modelling followed Gummerson (1986), but was modified to estimate hydrothermal time with germination rates through the 25% subpopulation.

STATISTICAL ANALYSIS

Seedling trait ordination

Given limited sample size, we used ordination to minimize path model complexity and capture seedling trait variation along a single axis per water treatment and harvest time. Prior to principal components analyses (PCA), several traits were transformed to reduce outlier influence (Table 1), and traits were relativized by standard deviates. From each of the four ordinations, we retained the first, most explanatory PCA axis (PC1) for path models. PCAs were performed in PC-ORD v.6.11 (McCune & Mefford 2011).

Path analysis

We used path analysis (reviewed by Kline 2011) to simultaneously test relationships between traits, life stage transition probabilities and cumulative first-year survival. Rather than utilizing a series of separate regressions, path analysis allowed us to specify and simultaneously quantify all hypothesized relationships, including indirect effects of traits on cumulative survival mediated through different life stages. Path coefficients are partial regression coefficients and can be interpreted similarly to simple and multiple regressions.

We initially specified two possible models. First, a full model represented the possibility that all four life stage transitions and concurrent traits influence cumulative survival (Fig. 1a). However, our first hypothesis suggests that a simpler model including only germination and emergence processes is sufficient to predict recruitment. Therefore, we also proposed a nested, reduced model including only these stages as predictors of cumulative survival (Fig. 1b). If our first hypothesis was supported, we would expect the reduced model to fit the data, to retain a high coefficient of variation for cumulative survival (despite exclusion of seedling stages) and to observe significant path coefficients for specified relationships. While we sought to directly compare fit and explanatory ability between full and reduced models, the full model did not fit for reasons external main hypotheses (discussed below). However, we were able to independently examine and draw inferences about hypotheses from reduced models.

All path models were estimated with Bayesian techniques using Markov Chain Monte Carlo (10 000 iterations from two MCMC chains) in MPlus v.6.12 (Muthén & Muthén 1998–2011). Bayesian estimation does not depend on asymptotic theory and was preferred given small sample size (Lee 2007). Missing values comprised <1% of the total data set and were assumed missing at random, allowing estimation under the TYPE = MISSING command in MPlus (Asparouhov & Muthén 2010). We report two goodness-of-fit metrics for models, including posterior predictive *P*-values (PPP, with values near 0·5 indicating good fit and extremes near 0 or 1 suggesting implausibility) and 95% credibility intervals for the difference between observed and replicated chi-square values (CI_{95}) (Asparouhov & Muthén 2010).

For reduced models, we report direct and indirect effects. We also directly compared unstandardized path coefficients of germination and emergence on cumulative survival. After running separate models for each water treatment, we compared unstandardized path coefficients between treatments by running an unconstrained model in which parameters were estimated simultaneously and freely for both treatments, testing for significantly different paths between treatments (α = 0·05). To identify sources of misspecification in the full model and inform future hypothesis testing of trait influence across seed and seedling stages, we examined observed (i.e. sample) correlation/covariance matrices and residual correlation matrices for all variables in the full model. Residual correlations (r_r) are the differences between observed and model-implied (i.e. estimated) correlations, with large values suggesting that the hypothesized model structure failed to capture some relationship between two variables (Kline 2011).
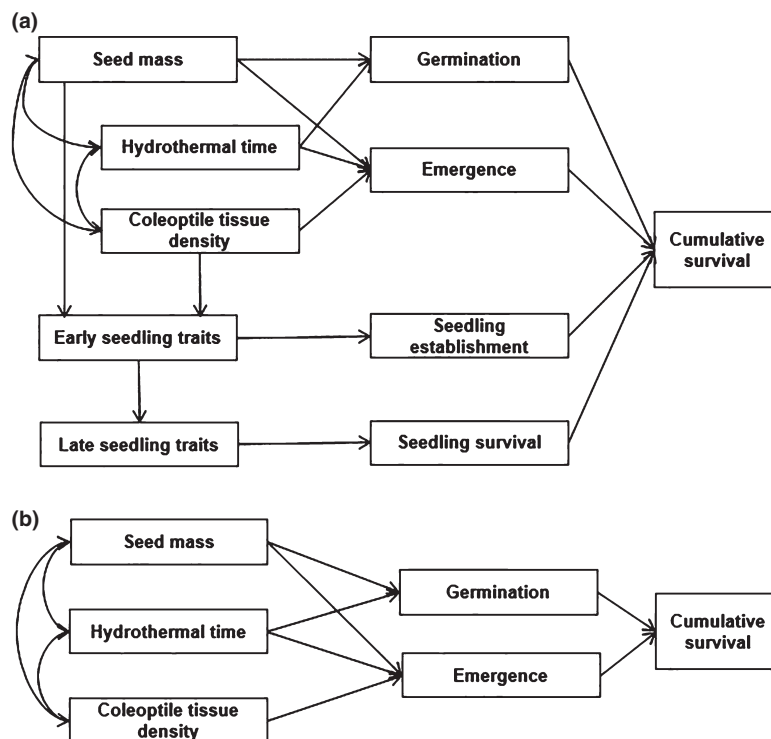
Fig. 1. (a) Full model and (b) hypothesized reduced model showing tested relationships between functional traits, mediating life stage transitions and cumulative survival. The reduced model represents the hypothesis that earlier life stages are sufficient to explain variation in recruitment.

Results

ORDINATION

Under the non-watered treatment, 43% and 36% of the variation in seedling traits were explained by PC1 for early and late harvests, respectively. Under the watered treatment, 45% and 48% of seedling trait variation were explained by PC1 for early and late harvests, respectively (Table S2; scores in Appendix S1, Supporting information). For early seedlings under both treatments, PC1 represented a tissue economics spectrum with positive loadings of LDMC, root mass, and root length and negative loadings of SLA and SRL. At the later seedling stage, PC1 represented a similar leaf economics spectrum but association with root traits shifted, with positive loadings of LDMC and negative loadings of SLA, root mass and root length (Table S2, Supporting information).

PATH ANALYSIS

Reduced models

Under the non-watered treatment, the reduced model fit the data [PPP = 0·311; CI_{95} = (−15·79, 26·52)] and explained 91% of the variation in cumulative survival with just two of four life stage transitions (Fig. 2a). Both germination and emergence had significant positive effects on cumulative survival, although the effect of emergence was greater (unstandardized $\rho_{csur·emg}$-$\rho_{csur·ger}$ = 0·51, $P < 0.001$). Approximately 16% of the variation in germination and 18% of variation in emergence were explained

by functional traits. Seed mass was positively associated with germination and negatively but weakly related to emergence (unstandardized path coefficient $\rho_{emg·seed}$ = −0·17, $P = 0.06$). Coleoptile tissue density was negatively associated with emergence, and hydrothermal time was not a predictor of either transition. Coleoptile tissue density was the only trait with a significant total indirect effect on cumulative survival (Table 2).

Under the watered treatment, the reduced model also fit the data [PPP = 0·494; CI_{95}(−21·21, 20·91)] (Fig. 2b). In the reduced model, germination and emergence explained 92% of the variation in cumulative survival, and the effect of emergence was larger (unstandardized $\rho_{csur·emg}$-$\rho_{csur·ger}$ = 0·32, $P < 0.001$). Approximately 23% of the variation in germination and 10% of variation in emergence were explained by functional traits. Seed mass was positively associated with germination, and hydrothermal time had a positive but non-significant association with germination (unstandardized $\rho_{ger·ht}$ = 1·97, $P = 0.099$). Total indirect effects of all traits on cumulative survival were non-significant (Table 2). The only unstandardized direct effect which differed significantly between treatments was the path from germination to cumulative survival ($\rho_{non-watered}$-$\rho_{watered}$ = −0·09, $P = 0.04$) (Table 3).

Full models

The full model did not fit observed data under either non-watered [PPP = 0·012; CI_{95}(6·15, 74·92)] or watered treatments [PPP = 0·007; CI_{95}(8·67, 77·59)]. Full models did not fit, so we do not report path coefficients, but use
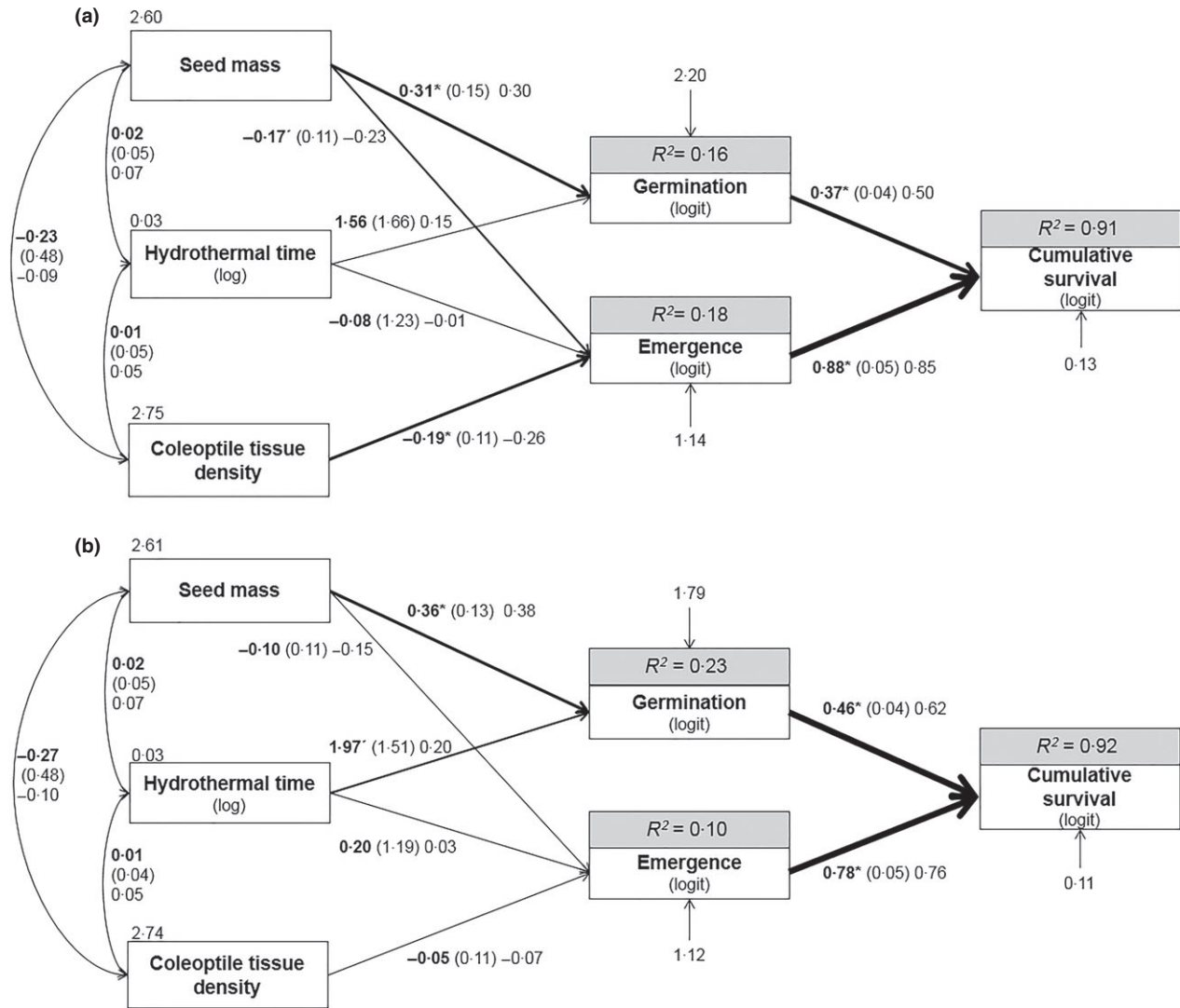
Fig. 2. Reduced path models showing relationships between traits, life stage transition probabilities and cumulative survival in (a) non-watered and (b) watered treatments. Path coefficients are partial regression coefficients, with arrows pointing from explanatory to response variables. Unstandardized path coefficients are bolded, followed by standard errors in parentheses and standardized estimates. Variances and residual variances are shown for exogenous and endogenous variables, respectively. Lines are weighted based on standardized estimates. *$P < 0.05$; †$P < 0.10$.

observed and residual correlations among full model variables to examine sources of misspecification. These data are reported in Table S3 (Supporting information) with important results highlighted below.

Large residual correlations ($r_r > 0.20$) under the non-watered full model occurred between late seedling traits and both CTD ($r_r = 0.39$) and seed mass ($r_r = -0.30$), and between seed mass and seedling survival ($r_r = 0.47$), early seedling traits and seedling survival ($r_r = 0.49$), CTD and seedling establishment ($r_r = -0.42$), late seedling traits and seedling establishment ($r_r = -0.38$), and between hydrothermal time and seedling establishment ($r_r = -0.29$). While life stage transition probabilities were assumed to be unrelated, a large residual also occurred between seedling establishment and survival ($r_r = 0.40$). Among all four life stages, emergence shared the highest

observed correlation with cumulative survival ($r = 0.79$). Observed correlations with germination ($r = 0.36$), seedling establishment ($r = 0.34$) and seedling survival ($r = 0.33$) were similar, though germination variance was threefold higher than variances of either seedling stage (Table S3, Supporting information), whose probabilities exceeded *c.* 0.60 across grasses (Appendix S1, Supporting information).

Under the watered full model (Table S3, Supporting information), residual correlations similarly suggested unanticipated relationships between CTD and late seedling traits ($r_r = 0.40$), seed mass and seedling survival ($r_r = 0.44$), early seedling traits and seedling survival ($r_r = 0.58$), CTD and seedling establishment ($r_r = 0.40$), and between late seedling traits and establishment ($r_r = -0.41$). An unanticipated association was also

Table 2. Standardized (St.) and unstandardized (Unst.) indirect effects of traits on cumulative survival in reduced path models. Total indirect effects account for effects on cumulative survival through multiple mediators. Standard errors and significance applies to unstandardized estimates

Predictor	Mediator	St. Estimate	Unst. Estimate	SE	*P*-value	95% Credibility interval	
Non-watered							
Seed Mass	Germination	0·149	0·114	0·057	0·023*	0·002	0·23
	Emergence	−0·193	−0·147	0·095	0·06[†]	−0·34	0·038
	Total	−0·044	−0·034	0·109	0·377	−0·247	0·179
Hydrothermal Time	Germination	0·075	0·583	0·623	0·169	−0·65	1·813
	Emergence	−0·008	−0·067	1·086	0·468	−2·142	2·097
	Total	0·067	0·516	1·188	0·334	−1·83	2·856
Coleoptile tissue density	**Total**	−0·224	−0·167	0·097	0·047*	−0·357	0·027
Watered							
Seed Mass	Germination	0·234	0·167	0·064	0·004*	0·042	0·297
	Emergence	−0·112	−0·08	0·083	0·158	−0·248	0·08
	Total	0·122	0·087	0·103	0·197	−0·116	0·288
Hydrothermal Time	Germination	0·126	0·915	0·705	0·099	−0·481	2·305
	Emergence	0·020	0·153	0·928	0·436	−1·644	2·001
	Total	0·003	1·068	1·12	0·17	−1·183	3·256
Coleoptile tissue density	**Total**	−0·050	−0·035	0·084	0·333	−0·201	0·135

*$P < 0.05$, [†]$P < 0.10$.

Table 3. Differences between unstandardized path coefficients (ρ) for non-watered and watered treatments under the reduced model

Path	$\rho_{non\text{-}watered} - \rho_{watered}$	SE	*P*-value	95% Credibility interval	
Seed mass → Germination	−0·06	0·20	0·39	−0·46	0·35
Hydrothermal time (log) → Germination	−0·41	2·25	0·43	−4·85	4·00
Seed mass → Emergence	−0·06	0·15	0·34	−0·37	0·23
Hydrothermal time (log) → Emergence	−0·29	1·70	0·43	−3·63	3·07
Coleoptile tissue density → Emergence	−0·14	0·15	0·18	−0·44	0·16
Germination → Cumulative survival	−0·09	0·05	0·04*	−0·19	0·01
Emergence → Cumulative survival	0·10	0·07	0·08[†]	−0·04	0·24

*$P < 0.05$, [†]$P < 0.10$.

suggested between emergence and seedling establishment ($r_r = 0.39$). Observed correlations were relatively small between cumulative survival and both seedling establishment and survival ($r = 0.37$ and $r = 0.17$, respectively) compared to germination and emergence ($r = 0.51$ and $r = 0.67$, respectively) (Table S3, Supporting information).

Discussion

DEMOGRAPHIC TRANSITIONS DRIVING RECRUITMENT VARIATION

Reduced models supported the first hypothesis that germination and emergence are key life stage transitions driving cross-species variation in recruitment probability. Regardless of soil moisture, germination and emergence probabilities explained over 90% of the variation in cumulative survival, with germination probabilities ranging from 0·15 to 1·0 and emergence probabilities ranging from 0·10 to

0·80 across grasses. Emergence probability explained most of the variation in cumulative survival, broadly expanding the idea that mortality among germinated seeds is not only a major recruitment bottleneck in some species (Hegarty & Royle 1978; James, Svejcar & Rinella 2011) but a critical driver of recruitment variation across species. While the potential importance of germination and emergence has long been recognized, partitioning these independent effects on cumulative survival across a range of native and introduced species offers a robust rationale for developing a new suite of management tools to increase germination and emergence probabilities of desirable species, with the greatest likely returns on improving emergence. In addition to refocusing trait and restoration species selection to consider germination/emergence processes (described below), developing new seed and soil preparation technologies that overcome these demographic barriers should translate to marked improvements to restoration outcomes (e.g. Madsen *et al.* 2012).

Contrary to our second hypothesis, water addition did not reduce the amount of variation in cumulative survival explained by early life stage transitions. Although emergence remained most influential, variation in germination probability became a slightly but significantly greater determinant of cumulative survival with water addition, suggesting that germination response to water addition may be more variable among species (e.g. Wainwright, Wolkovich & Cleland 2012) and/or less sensitive across species (e.g. Fay & Schultz 2009) relative to other life stage transitions. While our results suggest that emergence probability responded more positively to water addition than germination across varieties, overall, we found comparatively weak effects of water manipulation on these stages. The effect of watering in the field may have been insufficient to substantially alter germination and emergence barriers in the top soil layer (Leishman & Westoby 1994; Cipriotti *et al.* 2008), and trends should be tested under greater levels of manipulation. However, we increased total seasonal water amount by one-third, suggesting that managers may expect these larger recruitment patterns to hold across a range of precipitation scenarios.

LINKING RECRUITMENT VARIATION TO FUNCTIONAL TRAITS

Functional traits offered some insight into germination and emergence patterns, explaining 10–23% of the variation in these life stage transition probabilities under either watering treatment. While links have emerged between traits and abundance at larger scales (e.g. species distribution and community assembly (Cornwell & Ackerly 2010; Laliberté *et al.* 2012), efforts to link traits to underlying demographic patterns have been limited, focusing almost exclusively on a handful of well-studied leaf, wood, and architectural traits, which have comparatively explained as much as 30–50% of the variation in sapling and adult survival (Poorter *et al.* 2008; Martínez-Vilalta *et al.* 2010; Wright *et al.* 2010). Our work demonstrates a major need to broaden our understanding beyond these globally measured adult traits to identify how seed and seedling functional trait variation drive population dynamics.

Of the array of measured traits, coleoptile tissue density had the only significant effect on emergence and cumulative survival under non-watered conditions. Although few studies have examined coleoptile function, we might expect it to mirror leaf tissue economics such that survival under harsh conditions is mediated by either tolerance (via high tissue density) or escape (via low tissue density and rapid growth responses) (e.g. Kidson & Westoby 2000; González & Ayerbe 2011). Our results support the latter mechanism and suggest that water supplementation may alleviate some factor mediating this effect (e.g. by reducing soil penetration resistance).

Although seed mass is tied to many aspects of plant development and regeneration (Leishman *et al.* 2000), our results suggested weaker implications for emergence and recruitment success. By separating seed mass influences via path analysis, we found that while larger seed size tended to result in higher germination probabilities regardless of watering treatment, weak or negative effects on emergence resulted in a lack of net effect on cumulative survival. This trend corroborates a review of the literature finding little overall effect of seed mass on transition rates through emergence (Moles & Westoby 2004).

While hydrothermal time provides a comprehensive index of germination response to temperature and moisture, it was unable to capture germination variation in the field. Hydrothermal time had a marginal positive association with germination with watering, but this was likely driven by unexpectedly low germination probabilities among invasive annual grasses with low hydrothermal times. While low hydrothermal times should correspond to rapid germination, this observation suggests influence from unanticipated factors, for example bet-hedging dormancy mechanisms (Clauss & Venable 2000). This highlights a possible difficulty of predicting germination probabilities with a limited number of traits when several independent processes, such as hydrothermal accumulation and dormancy, may interact to obscure patterns of trait influence (e.g. Pérez-Fernández *et al.* 2000).

While traits captured a small portion of the variation in germination and emergence responses, the immediate benefit of utilizing functional traits as a unit of restoration was underlined by the extent of recruitment variation observed among varieties of the same species (Appendix S1, Supporting information). For example, while both varieties of *Elymus lanceolatus* were top recruiters, high-recruiting non-native *Agropyron desertorum* var. Hycrest II and native *Poa secunda* var. Opportunity exhibited at least 2- and 15-fold greater cumulative survival than conspecific varieties in ambient conditions, respectively. Ideally, trait-based frameworks will allow screening of high-performing varieties by a set of easily measured attributes. In agreement with our path model, *A. desertorum* var. Hycrest II was distinguished from conspecifics by lower coleoptile tissue density while both *E. lanceolatus* varieties also exhibited below-average values, despite vastly different seedling traits. However, *Poa secunda* var. Opportunity exhibited intermediate coleoptile tissue density and was not distinguished from conspecifics by seed mass. Given the complexity of ecological factors which may influence germination and emergence, traits should be utilized as guidelines for restoration rather than strict predictors of performance. However, while traits measured here provide some foundation to explain recruitment probabilities, a more comprehensive understanding of functional variation is clearly needed for trait-based recruitment frameworks to reach full potential.

A major challenge facing trait-based recruitment frameworks will thus be to incorporate the necessary level of trait resolution to capture multiple dimensions of germination and emergence response to environmental filters.

Drawing upon progress in seed and crop sciences, areas of future exploration include traits influencing seed susceptibility to pathogens and predators (e.g. seed coat hardness or seed chemical defences (reviewed by Dalling *et al.* 2011), dormancy potential (e.g. seed area to mass ratio or physiological controls (Dalling *et al.* 2011; Gardarin, Dürr & Colbach 2011), seed response to drying (e.g. seed water retention (Pérez-Fernández *et al.* 2000), and the survival and emergence of germinated seeds (e.g. morphology and physiology of early shoots and radicles (Gardarin, Dürr & Colbach 2010; González & Ayerbe 2011). To more fully link variation in demography to functional traits, our collective line of questioning must extend to the array of traits influencing seed and seedling interactions with below-ground environments.

REFINING A TRAIT-BASED RECRUITMENT FRAMEWORK

Additional considerations for the development of trait-based frameworks were highlighted by unexpected relationships in the full model. Firstly, while we attempted to account for trait collinearity, full model structure failed to capture relationships among traits of different stages. We expected ontogenetic conservation of tissue function between the coleoptile and the late seedling stage (e.g. Butterfield & Briggs 2011), but while this bivariate association was observed, it was not mediated through early seedling traits as hypothesized. Although early and late seedling trait axes represented similar leaf trait spectra (Table S2, Supporting information), they were not correlated (Table S3, Supporting information), suggesting that some key aspects of seedling function (root mass, SRL) may shift over short time scales during early recruitment. Furthermore, residual correlations suggested that several hypothesized relationships between traits and life stage transitions were misaligned (Table S3, Supporting information). Coleoptile tissue density was negatively associated with both emergence and establishment probabilities, while high LDMC and large roots at early establishment were positively associated with post-establishment survival, but not establishment. Collectively, this evidence underscores the importance of considering the timing of trait measurement and influence in recruitment frameworks.

In multispecies comparisons, it is also important to consider the role of phylogeny, since observed associations between traits and fitness could be a result of spurious correlations among closely related species. Unfortunately, our study does not lend itself to evolutionary interpretation given the difficulty of estimating relatedness among multiple varieties of the same species (some being artificially selected genotypes) (Stone, Nee & Felsenstein 2011). However, high variation among varieties of the same species and genus suggests a non-critical role for phylogeny in this study's conclusions (Appendix S1, Supporting information).

CONCLUSIONS AND IMPLICATIONS FOR MANAGEMENT

Our ability to intervene and mitigate ecosystem degradation across the globe is fundamentally tied to our ability to manage plant recruitment. For the first time, this study develops and tests a framework linking variation in key life stage transitions to variation in seed and seedling functional traits to identify drivers of recruitment outcomes. This framework and line of questioning moves the focus of applied plant functional trait work beyond trait influence on growth, resource acquisition and survival of established plants and towards a focus on how trait variation may influence recruitment following disturbance. In this system, 90% of the variation in seed to seedling survival among grasses was explained by germination and emergence, with candidate restoration species showing an eightfold variation in emergence probability, with up to sixfold variation among varieties of the same species. We identified a subset of traits driving a portion of the variation, but there are clear and compelling opportunities to greatly extend this understanding in this system and to begin identifying general global patterns of how trait variation drives variation in seedling recruitment.

While the need to move beyond species and towards a trait-based understanding of community assembly and ecosystem services has been widely acknowledged in the research community (Funk *et al.* 2008; Cadotte, Carscadden & Mirotchnick 2011) and is a logical concept for many managers and management situations (Garnier & Navas 2012), in practice, this shift has been slow to non-existent. A major constraint to adoption is that researchers and managers only understand a fraction of the traits driving critical recruitment outcomes. Stimulating this line of inquiry through cooperative research and management efforts and filling these large knowledge gaps will be essential to make meaningful practical advances in the ability of managers to use trait information to facilitate recovery of degraded ecosystems.

Acknowledgements

Data accessibility

Study species, trait data (species means and standard deviations) and life stage transition probabilities (species means and standard deviations) are available as online supporting information.

References

Abella, S.R., Newton, A.C., Fernandez-Bernal, A. & De La Rosa, M. (2009) *A Systematic Review of Species Performance and Treatment Effectiveness for Revegetation in the Mojave Desert, USA. Arid Environments*

and Wind Erosion. Nova Science Publishers, Inc., Hauppauge, NY, USA, 45–74.

Allen, P.S., Meyer, S.E. & Khan, M.A. (2000) *Hydrothermal Time as a Tool in Comparative Germination Studies, Seed Biology: Advances and Applications*, pp. 401–410. CABI Publishing, Wallingford, UK.

Andrews, M., Douglas, A., Jones, A.V., Milburn, C.E., Porter, D. & McKenzie, B.A. (1997) Emergence of temperate pasture grases from different sowing depths: importance of seed weight, coleoptiele plus mesocotyl length and shoot strength. *Annals of Applied Biology*, **130**, 549–560.

Asparouhov, T. & Muthén, B.O. (2010) Bayesian analysis using Mplus. Technical appendix. Los Angeles: Muthén & Muthén. www.statmodel. com

Bai, Z.G., Dent, D.L., Olsson, L. & Schaepman, M.E. (2008) Proxy global assessment of land degradation. *Soil Use and Management*, **24**, 223–234.

Butterfield, B.J. & Briggs, J.M. (2011) Regeneration niche differentiates functional strategies of desert woody plant species. *Oecologia*, **165**, 477–487.

Cadotte, M.W., Carscadden, K. & Mirotchnick, N. (2011) Beyond species: functional diversity and the maintenance of ecological processes and services. *Journal of Applied Ecology*, **48**, 1079–1087.

Cipriotti, P.A., Flombaum, P., Sala, O.E. & Aguiar, M.R. (2008) Does drought control emergence and survival of grass seedlings in semi-arid rangelands?: an example with a Patagonian species. *Journal of Arid Environments*, **72**, 162–174.

Clarke, P.J. & Davison, E.A. (2004) Emergence and survival of herbaceous seedlings in temperate grassy woodlands: recruitment limitations and regeneration niche. *Austral Ecology*, **29**, 320–331.

Clauss, M.J. & Venable, D.L. (2000) Seed germination in desert annuals: an empirical test of adaptive bet hedging. *The American Naturalist*, **155**, 168–186.

Cornwell, W.K. & Ackerly, D.D. (2010) A link between plant traits and abundance: evidence from coastal California woody plants. *Journal of Ecology*, **98**, 814–821.

Crist, T.O. & Friese, C.F. (1993) The impact of fungi on soil seeds: implications for plants and granivores in a semiarid shrub-steppe. *Ecology*, **74**, 2231–2239.

Dalling, J.W., Davis, A.S., Schutte, B.J. & Elizabeth Arnold, A. (2011) Seed survival in soil: interacting effects of predation, dormancy and the soil microbial community. *Journal of Ecology*, **99**, 89–95.

Doust, S.J., Erskine, P.D. & Lamb, D. (2008) Restoring rainforest species by direct seeding: tree seedling establishment and growth performance on degraded land in the wet tropics of Australia. *Forest Ecology and Management*, **256**, 1178–1188.

Engelbrecht, B.M.J., Kursar, T.A. & Tyree, M.T. (2005) Drought effects on seedling survival in a tropical moist forest. *Trees*, **19**, 312–321.

Fay, P.A. & Schultz, M.J. (2009) Germination, survival, and growth of grass and forb seedlings: effects of soil moisture variability. *Acta Oecologica*, **35**, 679–684.

Fenner, M. & Thompson, K. (2005) *The Ecology of Seeds*. Cambridge University Press, Cambridge, UK.

Funk, J.L., Cleland, E.E., Suding, K.N. & Zavaleta, E.S. (2008) Restoration through reassembly: plant traits and invasion resistance. *Trends in ecology & evolution*, **23**, 695–703.

Ganade, G. & Westoby, M. (1999) Seed mass and the evolution of early-seedling etiolation. *The American Naturalist*, **154**, 469–480.

Gardarin, A., Dürr, C. & Colbach, N. (2010) Effects of seed depth and soil aggregates on the emergence of weeds with contrasting seed traits. *Weed Research*, **50**, 91–101.

Gardarin, A., Dürr, C. & Colbach, N. (2011) Prediction of germination rates of weed species: relationships between germination speed parameters and species traits. *Ecological Modelling*, **222**, 626–636.

Garnier, E. & Navas, M.-L. (2012) A trait-based approach to comparative functional plant ecology: concepts, methods and applications for agroecology, A review. *Agronomy for Sustainable Development*, **32**, 365–399.

Gómez-Aparicio, L. (2008) Spatial patterns of recruitment in Mediterranean plant species: linking the fate of seeds, seedlings and saplings in heterogeneous landscapes at different scales. *Journal of Ecology*, **96**, 1128–1140.

González, Á. & Ayerbe, L. (2011) Response of coleoptiles to water deficit: growth, turgor maintenance and osmotic adjustment in barley plants (Hordeum vulgare L.). *Agricultural Sciences*, **02**, 159–166.

Gummerson, R.J. (1986) The effect of constant temperatures and osmotic potentials on the germination of sugar beet. *Journal of Experimental Botany*, **37**, 729–741.

Hardegree, S.P., Flerchinger, G.N. & Van Vactor, S.S. (2003) Hydrothermal germination response and the development of probabilistic germination profiles. *Ecological Modelling*, **167**, 305–322.

Hegarty, T.W. & Royle, S.M. (1978) Soil impedance as a factor reducing crop seedling emergence, and its relation to soil conditions at sowing, and to applied water. *Journal of Applied Ecology*, **15**, 897–904.

James, J.J., Svejcar, T.J. & Rinella, M.J. (2011) Demographic processes limiting seedling recruitment in arid grassland restoration. *Journal of Applied Ecology*, **48**, 961–969.

Kahmen, S. & Poschlod, P. (2008) Does germination success differ with respect to seed mass and germination season? Experimental testing of plant functional trait responses to grassland management. *Annals of Botany*, **101**, 541–548.

Kattge, J., Diaz, S., Lavorel, S., Prentice, I., Leadley, P., Bönisch, G. *et al.* (2011) TRY–a global database of plant traits. *Global change biology*, **17**, 2905–2935.

Keddy, P.A. (1992) A pragmatic approach to functional ecology. *Functional Ecology*, **6**, 621–626.

Kidson, R. & Westoby, M. (2000) Seed mass and seedling dimensions in relation to seedling establishment. *Oecologia*, **125**, 11–17.

Kline, R.B. (2011) *Principles and Practice of Structural Equation Modeling*. Guilford Press, New York, NY.

Laliberté, E., Shipley, B., Norton, D.A. & Scott, D. (2012) Which plant traits determine abundance under long-term shifts in soil resource availability and grazing intensity? *Journal of Ecology*, **100**, 662–677.

Lee, S.-Y. (2007) *Structural Equation Modeling: A Bayesian Approach*. John Wiley & Sons, West Sussex, England.

Leishman, M.R. & Westoby, M. (1994) The role of seed size in seedling establishment in dry soil conditions – experimental evidence from semi-arid species. *Journal of Ecology*, **82**, 249–258.

Leishman, M.R., Wright, I.J., Moles, A.T. & Westoby, M. (2000) The evolutionary ecology of seed size. *Seeds: The Ecology of Regeneration in Plant Communities*, **2**, 31–57.

Levine, J.M., Vilà, M., Antonio, C.M.D., Dukes, J.S., Grigulis, K. & Lavorel, S. (2003) Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **270**, 775–781.

Madsen, M.D., Davies, K.W., Williams, C.J. & Svejcar, T.J. (2012) Agglomerating seeds to enhance native seedling emergence and growth. *Journal of Applied Ecology*, **49**, 431–438.

Martínez-Garza, C., Bongers, F. & Poorter, L. (2013) Are functional traits good predictors of species performance in restoration plantings in tropical abandoned pastures? *Forest Ecology and Management*, **303**, 35–45.

Martínez-Vilalta, J., Mencuccini, M., Vayreda, J. & Retana, J. (2010) Interspecific variation in functional traits, not climatic differences among species ranges, determines demographic rates across 44 temperate and Mediterranean tree species. *Journal of Ecology*, **98**, 1462–1475.

McCune, B. & Mefford, M.J. (2011) PC-ORD. Multivariate analysis of ecological data. Version 6.11. MjM Software, Gleneden Beach, OR, USA.

McGill, B.J., Enquist, B.J., Weiher, E. & Westoby, M. (2006) Rebuilding community ecology from functional traits. *Trends in ecology & evolution*, **21**, 178–185.

Millennium Ecosystem Assessment (2005) *Ecosystems and Human Well-Being: Desertification Synthesis*. World Resources Institute, Washington, DC.

Moles, A.T. & Westoby, M. (2004) Seedling survival and seed size: a synthesis of the literature. *Journal of Ecology*, **92**, 372–383.

Muthén, L.K. & Muthén, B.O. (1998–2011) *Mplus User's Guide*, Sixth Edition. Muthén & Muthén, Los Angeles, CA, USA.

Pearson, T.R.H., Burslem, D.F.R.P., Mullins, C.E. & Dalling, J.W. (2002) Germination ecology of neotropical pioneers: interacting effects of environmental conditions and seed size. *Ecology*, **83**, 2798–2807.

Pérez-Fernández, M.A., Lamont, B.B., Marwick, A.L. & Lamont, W.G. (2000) Germination of seven exotic weeds and seven native speciesin south-western Australia under steady and fluctuating water supply. *Acta Oecologica*, **21**, 323–336.

Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P. *et al.* (2013) New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany*, **61**, 167–234.

Poorter, L., Wright, S.J., Paz, H., Ackerly, D.D., Condit, R., Ibarra-Manríquez, G. *et al.* (2008) Are functional traits good predictors of demographic rates? evidence from five neotropical forests. *Ecology*, **89**, 1908–1920.

Pyke, D.A. (1990) Comparative demography of co-occurring introduced and native tussock grasses: persistence and potential expansion. *Oecologia*, **82**, 537–543.

Pyke, D.A., Wirth, T.A. & Beyers, J.L. (2013) Does seeding after wildfires in rangelands reduce erosion or invasive species? *Restoration Ecology*, **21**, 415–421.

Rebollo, S., Pérez-Camacho, L., García-de Juan, M.T., Rey Benayas, J.M. & Gómez-Sal, A. (2001) Recruitment in a Mediterranean annual plant community: seed bank, emergence, litter, and intra-and inter-specific interactions. *Oikos*, **95**, 485–495.

Reich, P.B., Wright, I.J., Cavender-Bares, J., Craine, J.M., Oleksyn, J., Westoby, M. & Walters, M.B. (2003) The evolution of plant functional variation: traits, spectra, and strategies. *International Journal of Plant Sciences*, **164**, S143–S164.

Ryser, P. (1993) Influences of neighbouring plants on seedling establishment in limestone grassland. *Journal of Vegetation Science*, **4**, 195–202.

Seabloom, E.W., Harpole, W.S., Reichman, O.J. & Tilman, D. (2003) Invasion, competitive dominance, and resource use by exotic and native California grassland species. *Proceedings of the National Academy of Sciences*, **100**, 13384–13389.

Stone, G.N., Nee, S. & Felsenstein, J. (2011) Controlling for non-independence in comparative analysis of patterns across populations within species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **366**, 1410–1424.

Wainwright, C.E., Wolkovich, E.M. & Cleland, E.E. (2012) Seasonal priority effects: implications for invasion and restoration in a semi-arid system. *Journal of Applied Ecology*, **49**, 234–241.

Wright, I.J. & Westoby, M. (1999) Differences in seedling growth behaviour among species: trait correlations across species, and trait shifts along nutrient compared to rainfall gradients. *Journal of Ecology*, **87**, 85–97.

Wright, S.J., Kitajima, K., Kraft, N.J.B., Reich, P.B., Wright, I.J., Bunker, D.E. *et al.* (2010) Functional traits and the growth–mortality trade-off in tropical trees. *Ecology*, **91**, 3664–3674.

Zeiter, M., Stampfli, A. & Newbery, D.M. (2006) Recruitment limitation constrains local species richness and productivity in dry grassland. *Ecology*, **87**, 942–951.

Supporting Information

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

Table S1. Grass species and varieties included in the study ($n = 47$).

Table S2. Pearson's correlations of seedling traits with the first PCA axis for each of four ordinations (2 treatments × 2 harvests).

Table S3. Observed, estimated and residual correlations and observed covariances for variables in the full model.

Appendix S1. Grass variety data (means and standard deviations), including seedlot viability, life stage transition probabilities, and seed and seedling trait data.

Fig. S1. Diagram of experimental design.