



Direct and indirect effect of seed size on seedling survival along an experimental light availability gradient

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ABSTRACT

Theoretical models of life-history strategies assume a positive relationship between seed size and subsequent offspring survival (seed size–survival relationship). There is limited empirical evidence, however, on how this relationship changes across environments or in relationship to seedling traits. Moreover, characterizing the dynamics of seedling recruitment under natural conditions is central to understanding how seedling recruitment may in turn affect large-scale ecological processes. Here, first-year seedling survival was monitored from emergence for 303 angiosperms species originated from alpine and sub-alpine meadows of the eastern Tibetan Plateau grasslands, across an experimental light gradient consisting of four treatments. We used linear models (LM) and phylogenetic generalized linear models (PGLM) to test for the seed size–survival relationship, and how this relationship differed across light treatments. We also used path analysis (PA) and phylogenetic confirmatory path analysis (PPA) to assess how seed size interacted with other seedling traits (seedling emergence time, specific leaf area, root: shoot biomass ratio, and biomass growth) to either directly or indirectly affect seedling survival. We found seed size to be positively associated with seedling survival only under low to medium light treatments only in LM (not in PGLM). PA and PPA revealed that the positive effect of seed size on seedling survival was indirect, mainly acting via biomass growth. Under low light, larger seeds exhibited greater biomass growth, which in turn increased seedling survival. In contrast, the direct effect of seed size on seedling survival is negative. In sum, the seed size–survival relationship appears to be both environmentally and phylogenetically dependent. The survival advantage of large seeds appears to be the result of their ability of higher biomass growth after emergence in low resource environments. However, our results also suggest there may be an underlying trade-off with larger seeds facing a lesser, but direct risk of increased mortality. The influence of environment on seedling traits, the interaction among traits, and phylogeny should be taking into consideration when modeling the dynamics of seedling recruitment.

1. Introduction

Theoretical models of life-history strategies assume that seedling survival rate of large-seed species will be higher during establishment

in stressful, low-resource environments because of their greater resource storage capacity (i.e., a positive seed size–survival relationship) (Rees, 1995; Leishman et al., 2000; Muller-Landau, 2003; Fenner and Thompson, 2005; Metz et al., 2010). This theoretical assumption has

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found support in numerous field and experimental studies (reviewed in Leishman et al., 2000; Fenner and Thompson, 2005). In contrast, in more favorable, high-resource environments, the advantage of large-seeded species should be reduced, as the mortality caused by resource shortages in small-seeded species will be mitigated (Metz et al., 2010). Previous experimental work has borne this prediction out, with large seeds only exhibiting a survival advantage under unfavorable conditions, e.g. light-limited environments (Grime and Jeffrey, 1965; Hutchinson, 1967; Rose, 2000) and dry conditions (Leishman and Westoby, 1994). Our understanding of how seed size–survival relationship is affected by resource availability remains limited, however, because it has only been studied in a small number of species (less than 44) across sharply contrasting environments (reviewed in Leishman et al., 2000; Fenner and Thompson, 2005). How these dynamics play out at a community level across a realistic resource gradient remains to be explored.

In addition to environmental influences, little is known about how seed size interacts with other seedling traits to influence seedling survival (Baraloto et al., 2005). It has been well documented that seed size associates with many important seedling traits such as emergence time (Zhang et al., 2014b), specific leaf area (Poorter and Rose, 2005), biomass allocation (Lloret et al., 1999; Limpens et al., 2014) and biomass growth (Bonfil, 1998; Quero et al., 2007). These traits have, in turn, been shown to influence seedling survival (Lloret et al., 1999; Verdú and Traveset, 2005; Limpens et al., 2014; Reich, 2014). Thus, there are several pathways through which seed size might influence seedling survival indirectly via these additional seed traits (Fig. 1). For instance, large seeds are generally related to large seedlings which in turn increase seedling survival through escaping size-dependent mortality (Quero et al., 2007; Limpens et al., 2014), and therefore, large seeds might influence seedling survival through an effect on biomass growth (pink lines in Fig. 1; Hypothesis I). Large seeds also generally increase more resource allocation to roots vs. shoots, and higher root allocation in turn contributes the survival of seedlings through higher water-use and nitrogen-use efficiency (Baker, 1972; Lloret et al., 1999), and therefore, large seeds might influence seedling survival through an effect on root: shoot biomass ratio (black lines in Fig. 1; Hypothesis II). Small seeds are known to germinate early (Zhang et al., 2014b) and early germination is known to provide a positive advantage for final biomass and the survival of individuals (Verdú and Traveset, 2005; Dubois and Cheptou, 2012), and therefore, small seeds might influence seedling survival through an effect on germination timing (gray lines in Fig. 1; Hypothesis III). In addition, small seeds are also known to have higher specific leaf area which is thought to contribute to the growth advantage but lower the survival of seedlings (Maranon and Grubb, 1993; Reich, 2014), and therefore, small seeds might influence seedling

survival through an effect on specific leaf area (orange line in Fig. 1; Hypothesis IV).

Phylogenetic signal is the trend for closely related species to share similar traits (Losos, 2008), and has been demonstrated to be common at broad phylogenetic scales for numerous plant functional traits, including seed-related traits such as seed size and germination timing (Norden et al., 2009; Zhang et al., 2014b). Thus, it is possible that associations between seed/seedling traits described above could simply be a reflection of their shared evolutionary history (Cadotte and Davies, 2016). To account for the potential biases of shared evolutionary history in seed-related traits, we tested for phylogenetic signal in seed/seedling traits, as well as, corrected for phylogenetic signal in our correlative analyses.

Sub-alpine and alpine meadows are among the ecosystems most sensitive to the constant land use and climate change (Mu et al., 2016; Zhang et al., 2017b; Li et al., 2018). Characterizing the dynamics of seedling recruitment under natural conditions is central to understanding how seedling recruitment may in turn affect large-scale ecological processes. Moreover, knowledge about the process of seedling recruitment in alpine ecosystems is crucial for conservation and management (Tasser et al., 2007; Hejman et al., 2009). Yet the empirical evidence about seed size–survival relationships is rare for sub-alpine and alpine meadows. In addition, light availability is an important factor in alpine meadow communities, and has been shown to influence competition dynamics and niche differentiation in community assembly (Zhang et al., 2014b; Liu et al., 2015). Here, the first-year seedling survival was monitored across an experimental light availability gradient in 303 species from alpine and sub-alpine meadows on the eastern Tibetan Plateau grasslands. Seed size, seedling emergence time, specific leaf area, root: shoot biomass ratio and biomass growth were also measured. With this data, we addressed the following questions: (1) does first-year seedling survival increase with increased seed size? (2) does the relative advantage of large-seeded species increase or decrease with light availability? and (3) does seed size influence seedling survival directly or indirectly via one or more additional seedling traits (see Fig. 1)?

2. Materials and methods

2.1. Study region

The study region is located on the northeastern verge of the Tibetan Plateau in China (longitude from 101°05′ to 104°40′E, and latitude from 32°60′ to 35°30′N, and altitude from 2500 to 4000; about 40 000 km²) which is mainly belonging to alpine climate (Fig. A.1), where the mean annual precipitation and temperature are from 450 to 780 mm which is mainly accumulated in summer and autumn, and from -2.0–3.0 °C, respectively. Generally, the growing season is from late May to early October. The grassland types mainly belong to alpine/subalpine meadow, and the dominated monocotyledons and dicotyledons are mainly from the families of Poaceae and Cyperaceae, and the families of Asteraceae, Gentianaceae, Ranunculaceae, Fabaceae, Polygonaceae, Saxifragaceae, and Scrophulariaceae, respectively.

The selected 303 species adopted in this research contain most of the species dominant in this region. At the maturing time in 2009, more than 20 plants (natural populations) for most of the species were included to collect seeds. After that, the seeds from different plants in the same species were pooled and air-dried at room temperature. The pooled seeds were then weighed and planted.

2.2. Field pot experiment

In this study, we monitored the dynamics of seedling survival in response to experimentally varied light conditions (including natural light) in the field under seasonally variable conditions and in local soil. The filed pot experiment was conducted at a broad and flat site with the

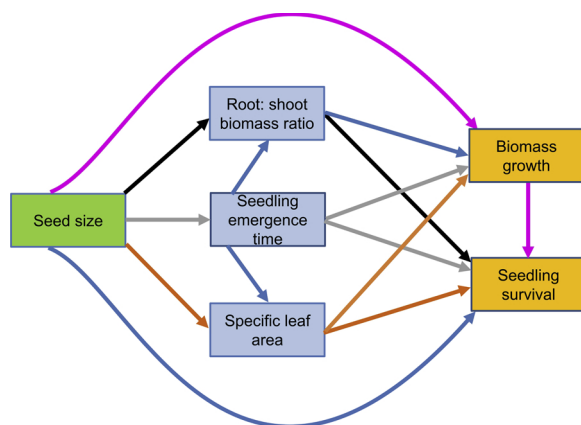


Fig. 1. The hypothetical pathways how seed size influences seedling survival through seedling emergence time, specific leaf area, root: shoot biomass ratio, and biomass growth.

sea level of 2980 m at the Research Station of Alpine Meadow and Wetland Ecosystems of Lanzhou University in the eastern Tibetan plateau, located in Hezuo, Gansu province of China (Fig. A.1), where the mean annual minimum temperature ranges from -10.0°C in January to 11.7°C in July with an average of 2.0°C , and the mean annual maximum temperature during the growing season ranges from 23.6 – 28.9°C with a short and cool summer (Fig. A.2). Mean annual precipitation over the last 35 years is 532 mm. Most of the local vegetation is from the species of *Elymus* sp., *Festuca* sp., *Roegneria* sp., *Scirpus* sp.

Seedling establishment was observed under four different light treatments: 100% unfiltered natural light (100% NL, mean PAR = $107,083.3 \mu\text{mol m}^{-2} \text{s}^{-1}$, SE = 1816.2), 65% filtered natural light (65% NL, mean PAR = $69,505.6 \mu\text{mol m}^{-2} \text{s}^{-1}$, SE = 1057.7), 32% filtered natural light (32% NL, mean PAR = $34,033.3 \mu\text{mol m}^{-2} \text{s}^{-1}$, SE = 1308.2), and 4.6% filtered natural light (4.6% NL, mean PAR = $4911.7 \mu\text{mol m}^{-2} \text{s}^{-1}$, SE = 755.9). Light levels in the three reduced light treatments (65% NL, 32% NL and 4.6% NL) were manipulated with plastic shade net. The photosynthetic active radiation (PAR) was measured at 1 cm above the soil surface for each light treatment. A Decagon Sunfleck Ceptometer (Pullman, Washington, D.C., US) was used for PAR recording. No significant difference in temperature was observed among four treatments (one way ANOVA: $F_{3,420} = 1.5$, $P = 0.222$; the means of 106 noondays from June to September and standard errors: 100% NL, $20.2 \pm 0.50^{\circ}\text{C}$; 65% NL, $19.4 \pm 0.51^{\circ}\text{C}$; 32% NL, $19.4 \pm 0.50^{\circ}\text{C}$; 4.6% NL, $18.8 \pm 0.43^{\circ}\text{C}$).

In order to ensure that seedling density (individuals/pot) was relatively constant across species (~12 seedlings per pot; the inner diameter of pot: 25 cm), the number of seeds planted per pot for each species was estimated based on germination percentage data from a previous field experiment (see Zhang et al., 2014a). Since germination percentages varied widely across species, the number of seeds planted also varied widely, ranging from 15 to 75 seeds per pot. For each treatment, we planted four replicate pots per species. In total, we planted between 240–1200 seeds per species (15 – 75 seeds \times 4 pots \times 4 treatments). Across all treatments, the final number of seedlings per pot was, on average, 11–12 seedlings (see Results for details).

For each pot, sandy soil from the Yellow River shore (where the seed bank was minimal; Ma et al., 2014) was used as top-soil, ~1 cm deep. The rest of the soil in each pot was taken from ~5–15 cm underground soil below the site area (see soil information in Table A.1). The 5–15 cm underground soil was dug and screened twice to mix it fully, and then covered with transparent plastic film and exposed to direct sunlight from July 2009 to May 2010 at the Research Station. There were two purposes for the above soil pretreatment: (1) to shelter from seed rain of 2009; (2) to germinate some seeds from soil seed bank. To prevent the emergence of additional seedlings from the seed bank in the bottom layer of soil, the two layers of soil in the pot were separated by grey cotton gauze (mesh: 1–3 mm) that stops the emergence of most dicotyledonous seedling, but does not stop the radicle of our planted seedlings from penetrating into the soil. Seeds were buried at 1-cm depth in the top sandy soil layer. We found it relatively easy to distinguish whether a seed germinated in the top sandy soil layer (originated from the seeds we added) or germinated in the soil layer from beneath the grey cotton gauze (originated from the seed bank). In high elevation, germination is mainly restricted to short periods in late spring and early summer, with many species germinating as soon as conditions are permissible (Körner, 2003). To avoid missing the growing season, our pot experiment was carried out as soon as practicality and conditions allowed on Jun. 4, 2010 and seedlings were monitored from emergence until Oct. 16, 2010 with a total of 19 weeks. Every seven days, the newly-emerged seedlings number and seedling mortality were recorded for each pot.

2.3. Phylogeny construction

Phyloomatic v3 was used to construct the composite phylogeny based

on the angiosperm megatree (R20091120.new) (Webb and Donoghue, 2005). The divergence time was estimated based on fossil data, and its relation with branch length was calculated with the function of the ‘blad’ in the program Phylocom 4.0 (Webb et al., 2008; Bell et al., 2010; Smith et al., 2010)

2.4. Seedling survival, seed size and seedling traits

Seedling survival was measured as the ratio of all surviving seedlings at the end of the growing season to all seedlings that emerged. To improve the estimation accuracy of seedling survival, we only included species where the mean number of individuals to emerge across pots in each treatment was ≥ 5 in our analysis.

Seed size was defined as the weight of the embryo, endosperm and seed coat or fruit coat, and not included accessories, such as wings, comas, pappus, elaiosomes, fruit flesh, etc. (Cornelissen et al., 2003). The weight was achieved by weighing 100 seeds for three times and then dividing the mean weight of three times by 100.

Seedling emergence time (days post planting) was calculated according to the following formula:

$$T = \Sigma(G_i \times i) / \Sigma G_i \quad (1)$$

where i is the number of days between seed sowing (day 0) and germination, and G_i is the number of seeds germinated on day i . Seedling emergence time was calculated separately for each pot within light treatments.

Specific leaf area was measured as the ratio of leaf area to leaf dry mass at the end of growing season. All leaves of each of six individuals in each of three pots within each treatment (if individual number in a pot was > 6 , six large individuals were chosen; if individual number was ≤ 6 , all individuals were chosen) were flattened and scanned. The three pots were picked out from the four pots in random for each species within each treatment. Leaf area was measured using the software ImageJ version 1.48v (Rasband, 2014). To measure leaf dry mass, root: shoot biomass ratio, and biomass growth (first-year individual mass), individuals were separated to leaves, stem and root, and then dried at 80°C to constant mass and weighed using a Sartorius balance accurate to 0.0001 g.

Prior to analyses, seed size (mg), seedling emergence time (days), specific leaf area ($\text{cm}^2 \text{mg}^{-1}$), root: shoot biomass ratio, and biomass growth (mg) were log-transformed to optimize distribution normality. Seedling survival rate was arcsine square root-transformed.

Seedling density (individuals/pot) measured at the end of the experiment was 11.0 (95%CI: 9.80–12.16) in 100% NL, 11.1 (9.94–12.27) in 65% NL, 12.9 (11.42–14.35) in 32% NL, and 10.1 (8.85–11.37) in 4.6% NL. We did not find a significant effect of seedling density on seedling survival rate across the light gradient ($df = 758$, $P = 0.8550$) or in each light treatment individually (100% NL: $df = 193$, $P = 0.3620$; 65% NL: $df = 195$, $P = 0.6220$; 32% NL: $df = 200$, $P = 0.1010$; 4.6% NL: $df = 164$, $p = 0.2520$; Table A.2). In addition, we did not find a significant effect of seedling density at the one-fourths point, mid-point and three-fourths point of the experiment (Table A.2). These results suggest that there was not a negative density dependent effect on seedling survival. For this reason, we did not include density in subsequent analyses.

2.5. Statistical analyses

One-way ANOVAs were used to analysis the effect of light treatments on seedling survival, seedling emergence time, specific leaf area, root: shoot biomass ratio and biomass growth. Comparisons between light treatments were corrected for the number of comparisons using a Tukey HSD test.

Both standard linear models (LM) and phylogenetic generalized linear models (PGLM; Revell and Harrison, 2008) were used to test the

effect of seed size, seedling emergence time, specific leaf area, root: shoot biomass ratio, and biomass growth on seedling survival. LM was performed with the “lm” function in the R package *stats* v3.2.2 (R Core Team, 2015). PGLM was performed with the “plgs” function in the R package *caper* v0.5 (Orme et al., 2011). With regard to the PGLM, a maximum likelihood framework was used to estimate Pagel's λ for the dependent variable.

To test for an effect of light treatment on the relationship between seedling survival and seed/seedling traits (seed size, seedling emergence time, specific leaf area, biomass growth, and root: shoot biomass ratio), analyses of co-variance (ANCOVA) were used. A significant interaction between treatment and seed trait would indicate that the effect of that seed trait on survival differs across light treatments. ANCOVA models were tested using the “lm” function of the R package *stats* v3.2.2 and the “Anova” function (type III) of the R package *car* v2.1-0 (R Core Team, 2015).

For each of four light treatments, both path analysis (PA) and phylogenetic confirmatory path analysis (PPA) were used to explore how seed size directly influences seedling survival or, through seedling emergence time, specific leaf area, root: shoot biomass ratio, and/or biomass growth, indirectly influences seedling survival (Shipley, 2002). A prior model was built, as shown in Fig. 1. Because there are so many priori pathways, we investigated all possible direct and indirect pathways in our path analysis. PPA integrates PGLS with the Δ -sep test developed by Shipley (2000) (see more details about PPA in von Hardenberg and Gonzalez-Voyer, 2013). The PA models were calculated using AMOS v5.0 (SPSS Inc.), and PPA using R v3.2.0 (R Core Team, 2015). The fitness of PA model to the data was evaluated with the maximum likelihood χ^2 goodness-of-fit test and goodness-of-fit index (GFI). For PPA, the model fitness was tested with Fisher's C goodness-of-fit test (Shipley, 2000; von Hardenberg and Gonzalez-Voyer, 2013).

Finally, Pagel's λ and Blomberg's K, two frequently used metrics, were used to test phylogenetic signal in seed size, seedling emergence time, specific leaf area, root: shoot biomass ratio, biomass growth, and seedling survival (Pagel, 1999; Blomberg et al., 2003). Both metrics evaluate the degree to which a trait displays phylogenetic signal according to a Brownian motion model of evolution. The “phylosig” function in the R package *phytools* v0.4-60 was used to estimate phylogenetic signal whose significance was evaluated using a randomization test (10 000 simulations) (Revell, 2012).

3. Results

In this study, we recorded 53,331 seedlings in total. Across all light treatments, seedling survival ranged from 0.15 to 1 (mean = 0.90). There is a significant effect of light treatment on seedling survival, with the highest seedling survival in 65% NL and the lowest in 4.6% NL (Fig. A.3a). The additional seedling traits were also affected, though in varying degrees, by light treatment. Seedling emergence time was significantly faster under low light conditions (Fig. A.3b). Specific leaf area was significantly higher under low light conditions (Fig. A.3c). Both root: shoot biomass ratio and biomass growth were highest in 65% NL and lowest in 4.6% NL (Fig. A.3b, A.3d). Across all 303 species, seed size ranged from 0.011–19.2 mg (Fig. A. 3f).

There were significant positive seed size–survival relationships in low light treatments (35% NL and 4.6% NL), but not in high light treatments (100% NL and 65% NL) (Table A.3, Fig. 2a). Seed size was positively associated with biomass growth in all treatment (Fig. 2c), seedling emergence only in high light (100% NL and 65% NL) (Fig. A.4o), and negatively associated with specific leaf area in all treatments except 4.6% NL (Fig. A.4 m). Many of the seedling traits were correlated with each other (Fig. A.4). Seedling emergence time was negatively associated with biomass growth and positively associated with specific leaf area in the 32% NL treatment (Fig. A.4 g, A.4n), and positively associated with root: shoot biomass ratio in all light treatments except

4.6% NL (Fig. A.4k). Specific leaf area was negatively associated with biomass growth in all treatments except 4.6% NL (Fig. A.4 h). Root: shoot biomass ratio was positively associated with seedling survival in all treatments except 32% NL (Fig. A.4d) and with biomass growth in the 65% and 32% NL treatments (Fig. A.4i). Biomass growth and seedling survival were positively associated in all treatments (Fig. 2b). See details of results in Table A.3.

Significant seed size–survival relationships were not found in any treatment in PGLM (Table A.4). Other results of PGLM were similar with LM. The details will be in the Table A.4.

Light treatments significantly changed the slope of the seed size–survival relationships. Light treatments also significantly changed the slope of other relationships, including relationships between seed size and other seedling traits except root: shoot biomass ratio, between seedling emergence time and specific leaf area, between specific leaf area and biomass growth, and between biomass growth and seedling survival (Figs. 2, A.4 and Table A.5). Low light treatments significantly increased the slope (from 0.19 to 0.65) and R^2 (from 0.05 to 0.72) of relationships between seed size (independent variable) and biomass growth (dependent variable) (Table A.3). See details of results in Tables A.3 and A.5.

According to the parameter values, our PA models fit data of four treatments very well (100% NL: $\chi^2 = 0.354$, $P = 0.902$, GPI = 0.998, RMSEAR = 0.000; 65% NL: $\chi^2 = 0.412$, $P = 0.521$, GPI = 0.999, RMSEAR = 0.000; 32% NL: $\chi^2 = 0.138$, $P = 0.711$, GPI = 1.000, RMSEAR = 0.000; 4.6% NL: $\chi^2 = 1.053$, $P = 0.305$, GPI = 0.998, RMSEAR = 0.019; see Table 1). Main results of PA (see Fig. 3 and Table A.6 for details) include: (1) seed size, via positively affecting biomass growth, indirectly affected seedling survival, and it resulted in a positive seed size–survival relationship; (2) seed size had directly negative effects on seedling survival, especially in 100% NL and 4.6% NL; (3) seed size firstly affected seedling emergence time, and then seedling emergence time, via affecting root: shoot biomass ratio and biomass growth, indirectly influenced seedling survival; (4) in general, root: shoot biomass ratio had positive effect on biomass growth and seedling survival, while seed size had no effect on root: shoot biomass ratio; (5) seed size negatively affected specific leaf area (except in the 4.6% NL treatment), while no direct effect of specific leaf area on seedling survival was identified; (6) in contrast to the linear models, specific leaf area positively affected biomass growth in the 4.6% NL treatment; (7) biomass growth positively affected seedling survival, and was also the primary avenue through which other traits indirectly affected survival; (8) the direct effect of biomass growth on survival increased with a decrease in light.

According to the parameter values, our PPA models also fit data of four treatments (see Table A.7). Although significant seed size–survival relationships were not found in PGLM, PPA also found that (1) the positive effect of seed size on seedling survival was indirect, mainly acting via biomass growth; (2) the direct effect of seed size on seedling survival was negative, especially in 4.6% NL. In PPA, the negative effect of seed size counteracted the most of the positive effect of seed size on seedling survival. In general, the results of PPA were similar to the results of PA. See detailed results in Fig. A.5 and Table A.8.

Significant phylogenetic signal was identified for seed size and all seedling traits in all four light treatments using Pagel's λ and Blomberg's K (Table A.9).

4. Discussion

We found that large-seeded species did have higher rates of first-year seedling survival, as predicted by theory, but only in low light. In more favorable, high-resource environments, such as high light availability, the advantage of species with larger seeds is reduced, as the mortality of species with smaller seeds due to resource shortages is mitigated. The dependency of this relationship on environmental factors is consistent with previous studies on smaller sets of species (e.g.,

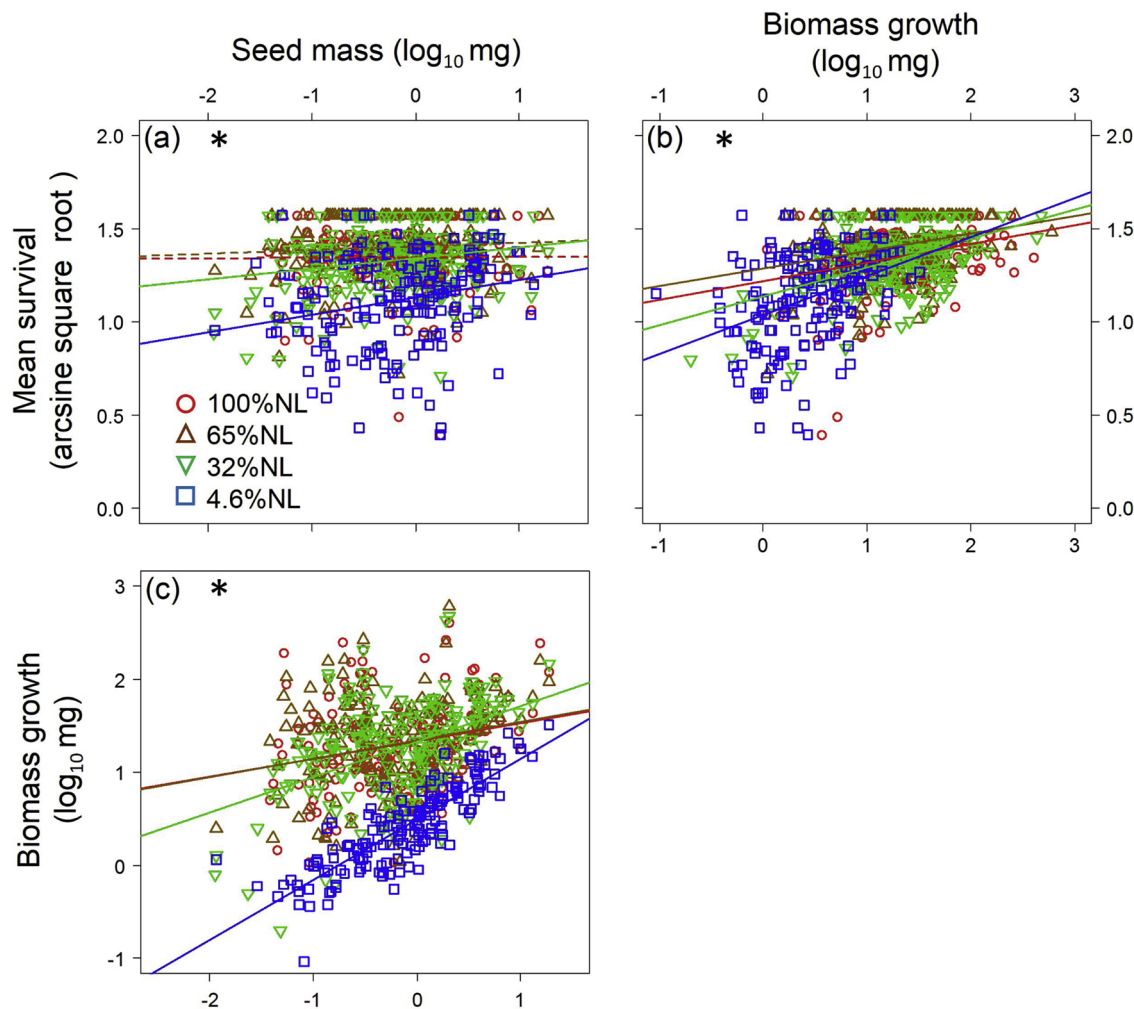


Fig. 2. Correlations among seed size, biomass growth and seedling survival along the light availability gradient. Significant relationships ($P < 0.05$) are denoted with solid lines. *: light treatments significantly changed the slope of the relationship; see details in Table A.5.

Grime and Jeffrey, 1965; Leishman and Westoby, 1994; Rose, 2000); that is, large seeds only exhibit a survival advantage under unfavorable conditions (e.g., low light environments, dry conditions). For example, in glasshouse study of 23 semi-arid species, Leishman and Westoby (1994) found that large seeds benefit seedlings establishment in dry soil conditions, but not wet soil conditions.

However, how do species with larger seeds counterpoise the quantitative advantage of species with smaller seeds in superior environments? Metz et al. (2010) found that lower variation in survival between years associated with larger seed size using 49 annual species based on a 7-year study under natural conditions. That is to say, a bet-hedging strategy is provided by larger seed size in temporally unpredictable environments. Consequently, it is suggested that simultaneously considering both the benefits of larger seed size (i.e., bet-hedging effect and higher survival rates) could improve our understanding

of plant life-history strategies. In the alpine/sub-alpine meadows and tropical forests, previous studies showed that large seeds tend to spread out germination across time (Norden et al., 2009; Zhang et al., 2014b), especially in natural light (vs. low light conditions) (Zhang et al., 2014b, a). In sum, large-seeded species could potentially use temporal bet-hedging strategies to compensate for the advantage of small-seeded species in high-resource environments.

Functional traits tend to covary according to the phylogenetic relationship among species (Losos, 2008). However, many studies assessing seed size–survival relationship have not accounted for possibility of phylogenetic signal (but see Moles and Westoby, 2004). In the only phylogenetic comparative study of seed size–survival relationship (112 species from 50 studies), Moles and Westoby (2004) reported that species with larger seeds had higher survival rates through early seedling establishment (1 week from emergence) than did small-seeded

Table 1
Summary of path analysis model fit.

Model	Model details	n	χ^2	d.f.	P	GFI	AGFI	RMSEAR index	BIC
a	100% NL	190	0.354	1	0.902	0.998	0.968	0.000	105.798
b	65% NL	190	0.412	1	0.521	0.999	0.985	0.000	105.353
c	32% NL	194	0.138	1	0.711	1.000	0.995	0.000	105.495
d	4.6% NL	156	1.053	1	0.305	0.998	0.953	0.019	102.050

n, sample size; GFI, goodness of fit index; AGFI, adjusted goodness of fit index; RMSEAR, Root mean square error of approximation; BIC, Akaike's Bayesian information criterion. See more results in Fig. 3 and Table A.6.

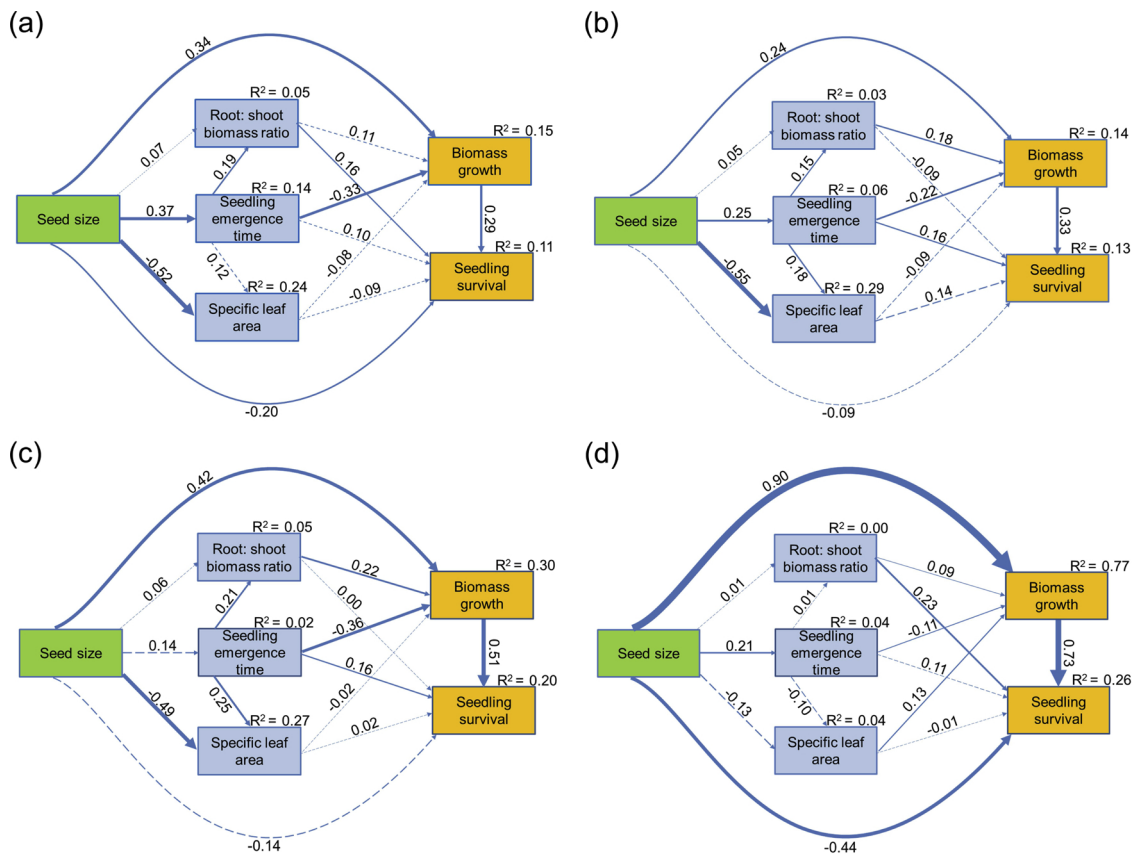


Fig. 3. Path analysis models showing the causal relationships among seed size (\log_{10} mg), seedling emergence time (\log_{10} days), specific leaf area (\log_{10} $\text{cm}^2 \text{mg}^{-1}$), root: shoot biomass ratio (\log_{10}), biomass growth (\log_{10} mg) and seedling survival (arcsine square root) along a light availability gradient (a, 100% NL; b, 65% NL; c, 32% NL; d, 4.6% NL). Numbers above arrows indicate path coefficients. Width of arrows indicates the strength of the causal influence. R² values represent the proportion of variance explained for each endogenous variable. Significant pathways are represented with solid lines ($P < 0.05$). See model fit summary in Table 1. See more results in Table A.6. See Fig. A.5, Tables A.7 and A.8 for phylogenetic confirmatory path analysis results.

species. In Tibetan Plateau grasslands, however, we found no seed size–survival relationships in one growing season when accounting for phylogenetic relatedness. One explanation for the difference between our study and Moles and Westoby (2004) is that seed size–survival relationships in Moles and Westoby (2004) were examined in the very early stage of seedling establishment, as opposed to the later stages we investigated. This could potentially lead to an overestimation of the seed size–survival relationship, as most of the advantage of larger seeds has been found to occur in the early stages of seedling establishment (reviewed in Leishman et al., 2000). Other possible explanations include: (1) Moles and Westoby (2004) did a meta-analysis of 50 studies and might have included a much wider range of seed sizes which could have magnified the effect; (2) it could be taxon sampling issue; our phylogenetic tree is dominated by herbaceous plants and some specific families (e.g., Asteraceae and Poaceae), the evolutionary and ecological history of which could potentially limit the effect of a seed size–survival trade-off.

The conflict between standard and phylogenetic comparative methods may result from these traits having a strong phylogenetic signal (Table A.9). This is to say that variation in seedling survival is better explained by species shared evolutionary history than by seed size in this community. Thus, the association between survival and seed size we observe in the standard comparative analysis is in fact spurious, an artifact of the phylogenetic signal in seed size itself. Once you account for the phylogenetic signal in survival and seed size, the residual variation between the two variables is uncorrelated. Nonetheless, this relationship through shared evolutionary history might still influence dynamics of seedling recruitment and even community assembly in communities. In addition to the standard and phylogenetic linear

regression analyses, the comparative path analyses can provide a deeper understanding of the seed size–survival relationships we observe. Although significant seed size–survival relationships were not found with the phylogenetic linear regression, both the standard and phylogenetic path analysis found that (1) seed size had indirectly positive effects on seedling survival, mainly acting via biomass growth, and (2) directly negative effects on seedling survival. In the phylogenetic path analysis, the negative effect of seed size counteracted the most of the positive effect of seed size on seedling survival, which helps to explain the non-significant seed size–survival relationship observed with the phylogenetic regression analysis.

In this study, one of the most interesting results is that the survival advantage of large seeds appears to be the result of their higher biomass growth after emergence. As shown in path analysis results (Figs. 3, A.5), the positive effect of seed size on seedling survival was indirect, acting via biomass growth, and this pathway was significant under all light conditions. Larger seedlings are more robust and then better able to escape size-dependent mortality and in turn increase seedling survival (Quero et al., 2007). In addition, it should be noted that the above pathway was strongest under low light. Seed size determined biomass growth of seedling to a great extent under low light, and greater biomass growth caused by larger seeds in turn largely increased seedling survival (Figs. 2, 3, and A.5). Quero et al. (2007) also found that the correlation between seed mass and seedling mass increases with a decrease in light in an intraspecific study. In sum, our results indicated the fundamental reasons why many previous studies found that large seeds only exhibit a survival advantage under unfavorable conditions (e.g. light-limited environments).

Another unusual and novel result we found was that the direct effect

of seed size on seedling survival was negative, which is contrary with previous studies (Leishman et al., 2000; Fenner and Thompson, 2005; Quero et al., 2007; Limpens et al., 2014). The studies on inter-specific seed size–survival relationships occurred frequently but are rare on community level, and additionally, they seldom involved multiple physiological and morphological traits of seedling and controlled for variation in these seedling traits (Baraloto et al., 2005). There are two possible explanations for this result. Firstly, at the beginning of seedling stage, larger radical of seedling from larger seed with low relative superficial area is harder to penetrate into the cracks of soil and reduces contact with the soil surface (Fenner and Thompson, 2005). Therefore, larger seedlings from larger seeds are easy to lodge and cannot effectively absorb water and mineral nutrients, and as a result, they may face higher risks. Secondly, larger seedlings or cotyledons from larger seeds may be more apparent and attractive to herbivores (Baraloto et al., 2005). In this study, however, we did not observe this case because there is a fence bounding the field station. It has been generally thought that larger seeds provide survival advantages in previous studies (reviewed in Fenner and Thompson, 2005), but our results suggested that seedlings from larger and smaller seeds may face different risks and they may alternately take survival advantages at different phases of seedling stages.

We found the complicated associations among seedling traits in this study. Besides biomass growth, seedling emergence time and root: shoot biomass ratio also mediated the seed size–survival relationship. In general, seed size was positively related with emergence timing, which is accordant with previous studies (Norden et al., 2009; Zhang et al., 2014b). When a seedling emerges will influence its future success as a plant (Donohue et al., 2010). Early seedling emergence time could be magnified into large differences in final biomass and reproduction of individuals (Verdú and Traveset, 2005), especially under competitive situations (Dyer et al., 2000). However, early-emerged seedlings may also face a higher risk of mortality caused by seasonal hazards (e.g. pathogens, predation and desiccation; Donohue et al., 2010). In addition, we found that late-emerged seedling can also increase seedling survival through higher root biomass allocation. Results of path analysis confirmed the both advantages and disadvantages of early seedling emergence (Figs. 3, A.5). Thus, small-seeded and large-seeded species may emerge at different times as a way to alleviate competition by taking advantage of different aspects of the seasonal environment.

Artificial grassland establishment is one of the fastest and most effective ways to restore the productivity of degraded grasslands (Ma et al., 2017). Breeding of high quality forage varieties is of great significance on the background of grassland degradation. In our study, many species (e.g., *Agrostis hugoniana*, *Deschampsia caespitosa*, *Medicago ruthenica*, *Poa angustifolia*, *Poa sinoglaucia*, *Roegneria breviglumis*) have the potential to be cultivated as forage varieties. Our research will provide basic data including seedling emergence rate and the dynamics of seedling recruitment for the cultivation of these forages.

5. Conclusions

In this study, we found that the seed size–survival relationship appears to be both environmentally and phylogenetically dependent. Moreover, we report two important findings about seed size–survival relationship: (1) the survival advantage of larger seeds appears to be the result of their ability of higher biomass growth after emergence in low resource environments, and (2) however, there may be an underlying trade-off with larger seeds facing a lesser, but direct risk of increased mortality. We also found that not only did all the seedling traits, including seedling survival, differ across the light treatments, the relationship between these traits differed as well. In sum, the influence of environment on seedling traits, the interaction among seedling traits, and phylogeny should be taking into consideration when modeling the dynamics of seedling recruitment. In addition, this field study has a reference for breeding of new herbage varieties and improving

cultivation treasures on the background of grassland degradation in Qinghai-Tibet plateau.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.agee.2019.05.009>.

References

- Baker, H.G., 1972. Seed weight in relation to environmental conditions in California. *Ecology* 53, 997–1010.
- Baraloto, C., Forget, P.M., Goldberg, D.E., 2005. Seed mass, seedling size and neotropical tree seedling establishment. *J. Ecol.* 93, 1156–1166.
- Bell, C.D., Soltis, D.E., Soltis, P.S., 2010. The age and diversification of the angiosperms re-revisited. *Am. J. Bot.* 97, 1296–1303.
- Blomberg, S.P., Garland, Jr T., Ives, A.R., Crespi, B., 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57, 717–745.
- Bonfil, C., 1998. The effects of seed size, cotyledon reserves, and herbivory on seedling survival and growth in *Quercus rugosa* and *Q. laurina* (Fagaceae). *Am. J. Bot.* 85 79–79.
- Cadotte, M.W., Davies, T.J., 2016. *Phylogenies in Ecology: A Guide to Concepts and Methods*. Princeton University Press, Princeton, New Jersey.
- Cornelissen, J.H.C., Lavorel, S., Garnier, E., Diaz, S., Buchmann, N., Gurvich, D., Reich, P., Ter Steege, H., Morgan, H.D., van der Heijden, M.G.A., Pausas, J.G., Poorter, H., 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Aust. J. Bot.* 51, 335–380.
- Donohue, K., Casas, R.R.D., Burghardt, L., Kovach, K., Willis, C.G., 2010. Germination, postgermination adaptation, and species ecological ranges. *Annu. Rev. Ecol. Evol. S.* 41, 293–319.
- Dubois, J., Cheptou, P.O., 2012. Competition/colonization syndrome mediated by early germination in non-dispersing achenes in the heteromorphic species *Crepis sancta*. *Ann. Bot.-London* 110, 1245–1251.
- Dyer, A.R., Fenech, A., Rice, K.J., 2000. Accelerated seedling emergence in interspecific competitive neighbourhoods. *Ecol. Lett.* 3, 523–529.
- Fenner, M., Thompson, K., 2005. *The Ecology of Seeds*. Cambridge University Press, Cambridge.
- Grime, J., Jeffrey, D., 1965. Seedling establishment in vertical gradients of sunlight. *J. Ecol.* 53, 621–642.
- Hejman, M., Klauisová, M., Hejmanová, P., Pavlů, V., Jones, M., 2009. Expansion of *calamagrostis villosa* in sub-alpine *nardus stricta* grassland: cessation of cutting management or high nitrogen deposition? *Agric., Ecosyst. Environ., Appl. Soil Ecol.* 129 (1), 91–96.
- Hutchinson, T., 1967. Comparative studies of the ability of species to withstand prolonged periods of darkness. *J. Ecol.* 55, 291–299.
- Körner, C., 2003. *Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems*, second ed. Springer-Verlag, Berlin.
- Leishman, M., Westoby, M., 1994. The role of large seed size in dry soil conditions: experimental evidence from semi-arid species. *J. Ecol.* 82, 249–258.
- Leishman, M.R., Wright, I.J., Moles, A.T., Westoby, M., 2000. The evolutionary ecology of seed size. In: Fenner, M. (Ed.), *Seeds: The Ecology of Regeneration in Plant Communities*. CABI, Wallingford, pp. 31–57.
- Li, J., Yang, C., Liu, X., Shao, X., 2018. Inconsistent stoichiometry response of grasses and forbs to nitrogen and water additions in an alpine meadow of the Qinghai-Tibet Plateau. *Agric., Ecosyst. Environ., Appl. Soil Ecol.* <https://doi.org/10.1016/j.agee.2018.12.016>. In press.
- Limpens, J., Egmond, E., Li, B., Holmgren, M., 2014. Do plant traits explain tree seedling survival in bogs? *Funct. Ecol.* 28, 283–290.
- Liu, Y., Mao, L., Li, J., Shi, G., Jiang, S., Ma, X., An, L., Du, G., Feng, H., 2015. Resource availability differentially drives community assemblages of plants and their root-associated arbuscular mycorrhizal fungi. *Plant Soil* 386, 341–355.
- Lloret, F., Casanovas, C., Peñuelas, J., 1999. Seedling survival of Mediterranean shrub-land species in relation to root: shoot ratio, seed size and water and nitrogen use. *Funct. Ecol.* 13, 210–216.

- Losos, J.B., 2008. Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecol. Lett.* 11, 995–1003.
- Ma, Z., Ma, M., Baskin, J.M., Baskin, C.C., Li, J., Du, G., 2014. Responses of alpine meadow seed bank and vegetation to nine consecutive years of soil fertilization. *Ecol. Eng.* 70, 92–101.
- Ma, Z., Zhang, C., Zhou, H., Yao, B., Zhao, X., 2017. Role of seed bank in establishment of single and mixed-sowing artificial grasslands of Tibetan Plateau. *Pol. J. Ecol.* 65, 334–345.
- Maranon, T., Grubb, P.J., 1993. Physiological basis and ecological significance of the seed size and relative growth rate relationship in mediterranean annuals. *J. Ecol.* 7, 591–599.
- Metz, J., Liancourt, P., Kigel, J., Harel, D., Sternberg, M., Tielbörger, K., 2010. Plant survival in relation to seed size along environmental gradients: a long-term study from semi-arid and Mediterranean annual plant communities. *J. Ecol.* 98, 697–704.
- Moles, A.T., Westoby, M., 2004. Seedling survival and seed size: a synthesis of the literature. *J. Ecol.* 92, 372–383.
- Mu, J., Zeng, Y., Wu, Q., Niklas, K.J., Niu, K., 2016. Traditional grazing regimes promote biodiversity and increase nectar production in Tibetan alpine meadows. *Agric. Ecosyst. Environ.* 233, 336–342.
- Muller-Landau, H.C., 2003. Seeds of understanding of plant diversity. *Proc. Natl. Acad. Sci. U. S. A.* 100, 1469–1471.
- Norden, N., Daws, M.I., Antoine, C., Gonzalez, M.A., Garwood, N.C., Chave, J., 2009. The relationship between seed mass and mean time to germination for 1037 tree species across five tropical forests. *Funct. Ecol.* 23, 203–210.
- Orme, D., Freckleton, R., Thomas, G., Petzoldt, T., Fritz, S., Isaac, N., Pearse, W., 2011. Caper: Comparative Analyses of Phylogenetics and Evolution in R. R Package Version 0.5.
- Pagel, M., 1999. Inferring the historical patterns of biological evolution. *Nature* 401, 877–884.
- Poorter, L., Rose, S.A., 2005. Light-dependent changes in the relationship between seed mass and seedling traits: a meta-analysis for rain forest tree species. *Oecologia* 142, 378–387.
- Quero, J.L., Villar, R., Marañón, T., Zamora, R., Poorter, L., 2007. Seed-mass effects in four Mediterranean *Quercus* species (Fagaceae) growing in contrasting light environments. *Am. J. Bot.* 94, 1795–1803.
- R Core Team, 2015. R: a Language and Environment for Statistical Computing. URL: R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
- Rasband, W.S., 2014. ImageJ, Version 1.48v. U.S. National Institutes of Health, Bethesda, Maryland, USA. <http://imagej.nih.gov/ij/>.
- Rees, M., 1995. Community structure in sand dune annuals: is seed weight a key quantity? *J. Ecol.* 83, 857–863.
- Reich, P.B., 2014. The world-wide ‘fast-slow’ plant economics spectrum: a traits manifesto. *J. Ecol.* 102, 275–301.
- Revell, L.J., 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* 3, 217–223.
- Revell, L.J., Harrison, A.S., 2008. PCCA: a program for phylogenetic canonical correlation analysis. *Bioinformatics* 24, 1018–1020.
- Rose, S.A., 2000. Seeds, Seedlings and Gaps-size Matters. A Study in the Tropical Rain Forest of Guyana. Ph.D. dissertation. Utrecht University, Tropenbos-Guyana series 9, Ipskamp, Enschede, Netherlands.
- Shipley, B., 2000. A new inferential test for path models based on directed acyclic graphs. *Struct. Equ. Model.* 7, 206–218.
- Shipley, B., 2002. Cause and Correlation in Biology: a User’s Guide to Path Analysis, Structural Equations and Causal Inference. Cambridge University Press, Cambridge, UK.
- Smith, S.A., Beaulieu, J.M., Donoghue, M.J., 2010. An uncorrelated relaxed-clock analysis suggests an earlier origin for flowering plants. *Proc. Natl. Acad. Sci. U. S. A.* 107, 5897–5902.
- Tasser, E., Walde, J., Tappeiner, U., Teutsch, A., Noggler, W., 2007. Land-use changes and natural reforestation in the Eastern Central Alps. *Agric. Ecosyst. Environ.* 118, 115–129.
- Verdú, M., Traveset, A., 2005. Early emergence enhances plant fitness: a phylogenetically controlled meta-analysis. *Ecology* 86, 1385–1394.
- von Hardenberg, A., Gonzalez-Voyer, A., 2013. Disentangling evolutionary cause-effect relationships with phylogenetic confirmatory path analysis. *Evolution* 67, 378–387.
- Webb, C.O., Donoghue, M.J., 2005. Phylomatic: tree assembly for applied phylogenetics. *Mol. Ecol. Notes* 5, 181–183.
- Webb, C.O., Ackerly, D.D., Kembel, S.W., 2008. Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* 24, 2098.
- Zhang, C., Liu, K., Qi, W., Ma, Z., Du, G., 2014a. Light-dependent associations of germination timing with subsequent life-history traits and maternal habitats for 476 angiosperm species of the eastern Tibetan Plateau grasslands. *Seed Sci. Res.* 24, 207–215.
- Zhang, C., Willis, C.G., Burghardt, L.T., Qi, W., Liu, K., Moura Souza-Filho, P.R., Ma, Z., Du, G., 2014b. The community-level effect of light on germination timing in relation to seed mass: a source of regeneration niche differentiation. *New Phytol.* 204, 496–506.
- Zhang, C., Ma, Z., Du, G., 2017a. Light-dependent associations of germination proportion with seed mass in alpine grasslands of the qinghai-tibet plateau. *Ecol. Eng.* 105, 306–313.
- Zhang, C., Willis, C.G., Klein, J.A., Ma, Z., Li, J., Zhou, H., Zhao, X., 2017b. Recovery of plant species diversity during long-term experimental warming of a species-rich alpine meadow community on the Qinghai-Tibet plateau. *Biol. Conserv.* 213, 218–224.