

LETTER

Functional trade-offs increase species diversity in experimental plant communities

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Abstract

Functional trade-offs have long been recognised as important mechanisms of species coexistence, but direct experimental evidence for such mechanisms is extremely rare. Here, we test the effect of one classical trade-off – a negative correlation between seed size and seed number – by establishing microcosm plant communities with positive, negative and no correlation between seed size and seed number and analysing the effect of the seed size/number correlation on species richness. Consistent with theory, a negative correlation between seed size and seed number led to a higher number of species in the communities and a corresponding wider range of seed size (a measure of functional richness) by promoting coexistence of large- and small-seeded species. Our study provides the first direct evidence that a seed size/number trade-off may contribute to species coexistence, and at a wider context, demonstrates the potential role of functional trade-offs in maintaining species diversity.

Keywords

Annual plants, coexistence, competition–colonisation trade-off, functional diversity, manipulation experiment, neutral theory, null models, seed size, species diversity.

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INTRODUCTION

Understanding the mechanisms that allow competing species to coexist in a community has long been a challenge for ecologists (Chesson 2000). This question is particularly challenging in the case of plants, as all plants compete for the same fundamental resources (light, water and nutrients). Nevertheless, observations in natural communities show that hundreds of plant species may coexist in one hectare of forest, and tenths of species may coexist in a square metre of grassland, all requiring the same resources (Silvertown 2004; Wilson *et al.* 2012).

Ecological theories have provided different explanations for this puzzle (Wright 2002; Amarasekare 2003; Silvertown 2004). One major explanation is trade-offs between different functional traits. Such trade-offs may contribute to species coexistence if relative fitness is affected by different traits and a beneficial change in one trait is associated with a detrimental change in another trait due to evolutionary constraints (Stearns 1989). Functional trade-offs may involve a wide spectrum of mechanisms (e.g., growth vs. reproduction, tolerance to shade vs. tolerance to drought, competitive vs. colonisation ability, etc.), but all mechanisms ultimately permit coexistence by reducing the likelihood that any one or a few species with particular traits will competitively exclude all other species in the community (Kneitel & Chase 2004).

For plants, a trade-off between seed size and seed number has been proposed as a major coexistence mechanism (Rees 1995; Rees *et al.* 1996; Leishman 2001; Turnbull *et al.* 2005; Muller-Landau 2010). According to this hypothesis, an individual plant may utilise its resources to produce either a small number of large seeds or a large number of small seeds (Smith & Fretwell 1974). Small-seeded species

are more prolific and therefore capable of reaching a larger number of vacant sites, whereas large-seeded species enjoy better competitive ability at the seedling stage and/or higher tolerance to environmental stress due to a larger maternal storage. Mathematical models demonstrate that such a trade-off allows a large number of competing species to stably coexist in a community (Rees & Westoby 1997; Geritz *et al.* 1999; Calcagno *et al.* 2006; Esther *et al.* 2008; Muller-Landau 2010). This trade-off also provides a possible explanation for another fundamental characteristic of plant communities – the enormous variation in seed size among coexisting plants with similar adult size (up to five orders of magnitude, Leishman *et al.* 1995; Jakobsson & Eriksson 2000). This variation requires attention because seed size is an important determinant of plant fitness (Saverimuttu & Westoby 1996; Leishman 2001; Moles & Westoby 2002, 2004b) and therefore, in the absence of any trade-off, coexisting species can be expected to show a convergence to some optimal seed size.

The recognition that a seed size/number trade-off provides potential explanations for two fundamental patterns in plant communities triggered numerous field studies that focus on the potential mechanisms behind this trade-off. These studies have provided important information about the magnitude of variation in seed size among coexisting species (Leishman *et al.* 1995; Jakobsson & Eriksson 2000), the correlation between seed size and seed number (Turnbull *et al.* 1999; Jakobsson & Eriksson 2000; Levine & Rees 2002), the potential advantages of large seeds (Westoby *et al.* 1992; Saverimuttu & Westoby 1996; Jakobsson & Eriksson 2000; Leishman 2001; Moles & Westoby 2002, 2004a), the effect of seed size on dispersal and colonisation abilities (Saverimuttu & Westoby 1996; Leishman 2001; Jakobsson & Eriksson 2003) and the relationship between seed size and abundance (Rees 1995; Eriksson

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& Jakobsson 1998; Guo *et al.* 2000; Levine & Rees 2002). However, the most fundamental hypothesis of the seed size/number trade-off, namely, that such a trade-off contributes to coexistence of species with variable seed sizes and to species richness in general has never been put to a direct experimental test.

Here, we provide an experimental test of this hypothesis. Specifically, we tested the basic prediction of the seed size/number trade-off, that a negative correlation between seed size and seed number among species increases species richness. We also tested the hypothesis that a seed size/number trade-off increases the likelihood of small-seeded species to persist in a community, thereby increasing the magnitude of among-species variation in seed size. An experimental test of this hypothesis is important because documenting variation in seed size among species in a community does not imply that the underlying mechanism is a seed size/number trade-off, even if seed size and number are negatively correlated.

Finally, we tested the hypothesis that the *mechanism* by which the seed size/number trade-off promotes coexistence is a trade-off between competitive ability and colonisation ability (i.e. a competition-colonisation trade-off, Tilman 1994). This hypothesis is a fundamental element in many models of the seed size/number trade-off (Geritz 1995; Leishman & Murray 2001; Levine & Rees 2002; Esther *et al.* 2008; Turnbull *et al.* 2008), but its applicability to real communities has been questioned (Turnbull *et al.* 1999; Coomes & Grubb 2003; Moles & Westoby 2004a).

We tested our hypotheses using a microcosm experiment focusing on annual plants representing red loamy soils of the coastal region in Israel. Specifically, we constructed artificial communities of annual species with a wide range (> 3 orders of magnitude) of seed sizes and seed numbers, manipulated the correlation between seed size and seed number within the communities and tested the effect of the experimentally induced size/number correlation on the fate of individual species and the number of species that persisted to

maturity. Further laboratory-controlled germination experiments were conducted to distinguish between environmental and intrinsic factors that could affect the observed responses.

METHODS

The experiment was conducted in a flat area of 1 ha allocated for this purpose at the Hebrew University Botanical Gardens. Within this area, 153 plots were built from plastic constructions that were set on the ground (Fig. 1). Each plot was 1 x 1 m in size and 0.5 m in height. The plots were filled with red loamy soil brought from a minimum depth of half metre in natural habitats of the study species to minimise seed bank. Nylon canvas was stretched underneath the plots to disconnect them from the ground (Fig. 1b).

Of the 153 plots, 63 were used as *community plots* and 90 were used as *reference plots* (Fig. 1b and c). Community plots were sown with seeds of 15 different species (Table 1) at a total density of 4476 seeds per 1 m². This sowing density resulted in seedling densities that were comparable to natural habitats of the study species. Sowing was limited to the inner 0.5 x 0.5 m of each plot, which was delimited by a grass blocker (Fig. 1b). The number of seeds sown from each species was chosen from a fixed set of abundance classes that approximates a log-normal distribution (2, 4, 8, 12, 16, 20, 24, 28, 32, 40, 48, 64, 128, 256, 512 seeds per species, Table 1). Three 'community treatments' were used for allocating species to the selected abundance classes: (1) Positive correlation between seed mass and seed number (nine replicated communities with Spearman correlation = 1, Table 1); (2) Negative correlation between seed mass and seed number (nine replicated communities with Spearman correlation = -1) and (3) Random allocation of the 15 abundance classes to the 15 species in 15 different communities under the constraints that each abundance class occurs only once in each row and once in each column of the 15 x 15 species x community matrix

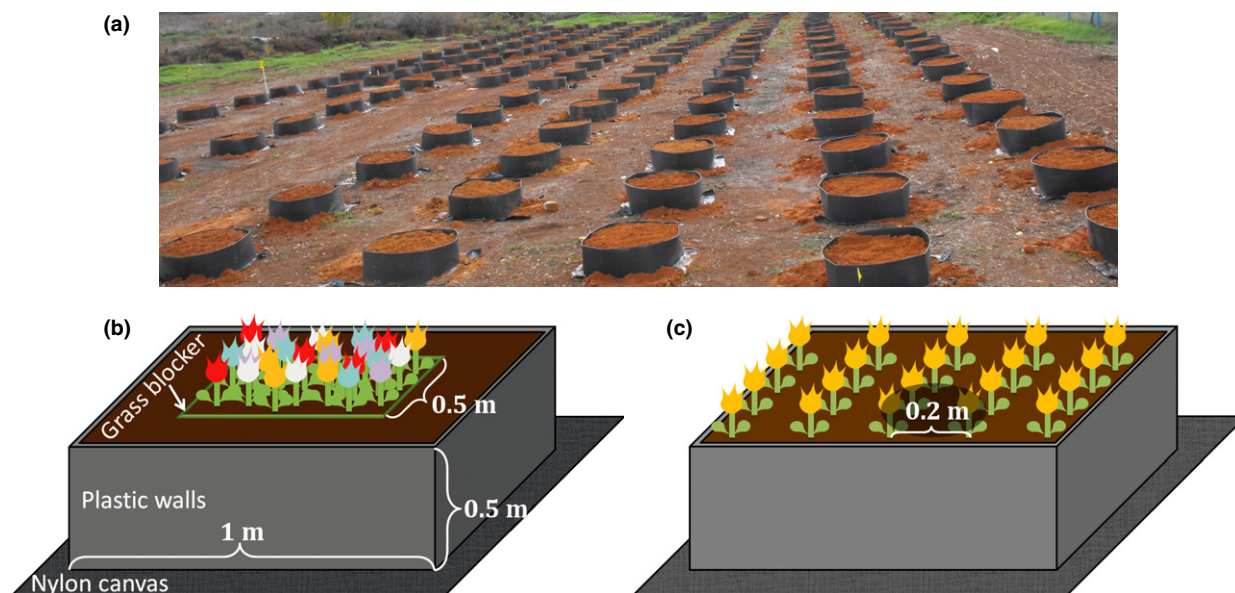


Figure 1 The experimental plots. (a) A general view of the experimental plots before the start of the experiment. (b) An illustration of a *community plot* sown with all 15 species in the middle quarter of the plot. (c) An illustration of a *reference plot* with a monoculture of isolated plants. Plots were built from plastic plates of 4.0 x 0.5 m that were folded to create a square plot of 1 x 1 m in area and 0.5 m in height. The grass blockers consist of plastic plates that were inserted to the ground to reduce the effective area of the plot to 0.5 x 0.5 m.

Table 1 List of the study species (annual herbaceous from the coastal plain flora representing a wide range of seed size), their seed mass and the number of seeds sown in each community treatment. Each column of numbers ranges from 2 to 512 and represents the assignment of the 15 abundance classes to the 15 species in the relevant community. The number of replicates of each community is given in bold at the bottom of the table

Species	Seed mass (mg)	Community treatment (Spearman ranking correlation between seed size and seed number):																
		r = 1	r = -1	Average r ≈ 0 for the 15 randomised communities														
<i>Lupinus palaestinus</i>	238.70	512	2	512	64	16	28	4	48	128	32	12	24	20	256	8	40	2
<i>Lupinus luteus</i>	58.81	256	4	40	512	32	64	28	12	20	8	2	128	24	4	256	16	48
<i>Vicia villosa</i>	30.66	128	8	16	20	512	32	2	24	40	48	4	12	64	8	28	256	128
<i>Raphanus raphanistrum</i>	3.44	64	12	28	48	12	512	64	4	24	16	32	256	8	128	2	20	40
<i>Trifolium purpureum</i>	1.60	48	16	12	8	20	256	512	16	2	64	24	28	128	40	48	4	32
<i>Rumex bucephalophorus</i>	0.55	40	20	256	28	40	4	16	512	48	20	8	2	32	64	24	128	12
<i>Trifolium tomentosum</i>	0.47	36	24	32	128	24	2	256	8	512	12	40	64	4	20	16	48	28
<i>Lotus halophilus</i>	0.37	28	28	2	4	8	48	128	256	64	512	16	40	12	24	32	28	20
<i>Silene colorata</i>	0.24	24	32	20	40	2	24	8	32	16	128	512	48	28	12	4	64	256
<i>Anthemis leucanthemifolia</i>	0.23	20	40	8	24	48	128	32	40	12	4	64	512	256	28	20	2	16
<i>Rostraria cristata</i>	0.10	16	48	128	2	4	8	20	28	256	40	48	32	512	16	64	12	24
<i>Silene palaestina</i>	0.10	12	64	48	32	28	16	12	64	8	256	128	20	2	512	40	24	4
<i>Papaver humile</i>	0.07	8	128	24	16	128	12	40	20	28	2	256	4	48	32	512	8	64
<i>Maresia pulchella</i>	0.05	4	256	4	256	64	20	48	128	32	24	28	16	40	2	12	512	8
<i>Campanula sulphurea</i>	0.02	2	512	64	12	256	40	24	2	4	28	20	8	16	48	128	32	512
Number of replicates		9	9	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

(three replicated sets of 15 randomised communities with mean Spearman correlation ≈ 0 in each set, Table 1). This randomisation procedure ensured that seed size and seed number will not be correlated, thus enabling optimal separation of the two factors in the statistical analyses and preventing the need to incorporate phylogenetic corrections – two major difficulties in analyses based on a small sample size. Hence, initial species richness, total abundance of all seeds of all species and the frequency distribution of seed abundance among species were similar for all experimental communities, but the assignment of the 15 species to the 15 abundance classes within each community was manipulated to create replicated communities with positive, negative and no correlation between seed size and seed number.

Table 1 provides full details on species abundances and the number of replicates used for each type of community. All plots were sown on February 3rd–4th 2009. In addition to natural precipitation, each plot received supplementary irrigation equivalent to 1-mm precipitation twice a week until the middle of April 2009 due to sparse days of rainfall during this period. Seedlings that emerged in each plot were identified and counted and seedlings of species that were not part of the experiment were removed. In addition, presence/absence of reproductive (flowering) individuals was determined for each species in each community at the end of the growing season (June 2009). The latter data were used to calculate species richness and frequency of occurrence of individual species. Thus, values of species richness and frequency of occurrence reported in all analyses refer to those species that reached the reproductive stage at the end of the experiment.

On the basis of the data on seed mass (Table 1), we also determined the range of seed sizes in each community plot. This range was used as a one-dimensional measure of ‘functional richness’ (Villegier *et al.* 2008) and allowed us to interpret our results in the wider context of recent theories about the relationships between functional traits, functional diversity and species diversity.

The reference plots (Fig. 1c) were established to provide data required for calibrating null models of species richness and frequency of occurrence (see below). These plots were sown with

seeds of a single species (six plots for each species, ~150–250 seeds per plot) and were thinned to densities of 20–25 seedlings per plot (a minimum distance of 20 cm between neighbouring plants) to minimise competitive interactions (Fig. 1c). Each seedling was monitored to determine whether it survived to reproduction (i.e., produced at least one flower), and the data obtained from the six plots of each species were pooled to determine probabilities of seedling survival at the absence of competitive effects. One species (*Lupinus luteus*) had only few seedlings in the whole experiment due to high seed dormancy and was therefore excluded from all analyses.

To evaluate whether our results can be attributed to a competition–colonisation trade-off, we compared the observed values of species richness and frequency of occurrence with corresponding predictions of null models assuming no competition. For each community plot j , the number of species expected to survive to the end of the growing season at the absence of competition was determined as:

$$S_j(\text{null}) = \sum_i \left(1 - m_i^{N_{i,j}}\right)$$

where $S_j(\text{null})$ is the number of species expected to survive in plot j at the absence of competitive interactions, $N_{i,j}$ is the number of seedlings of species i emerged in plot j and m_i is mortality probability of seedlings of species i at the absence of competition. The expression in parentheses $\left(1 - m_i^{N_{i,j}}\right)$ indicates the probability of a species with N seedlings to be present in community j at the end of the growing season under the assumption that mortality events are independent and occur with probability m . Values of m_i were obtained from the data collected in the reference plots. As we did not have the abundance of the species, we used a similar approach to calculate for each species a null expectation for the frequency of occurrence in a set of P plots as:

$$F_i(\text{null}) = \frac{\sum_{j=1}^P \left(1 - m_i^{N_{i,j}}\right)}{P}$$

where $F_i(\text{null})$ is the expected frequency of occurrence of species i at the absence of competitive interactions and P is the number of

community plots for which the expected frequency of occurrence is estimated. A comparison of species richness and species' frequencies of occurrence in the experimental communities with the corresponding null-model predictions allowed us to assess the role of competitive interactions in determining these two variables.

In addition to the main experiment, we conducted germination tests to evaluate the viability of the seeds used in the experiment under controlled laboratory conditions. Five Petri agar (1%) dishes with ten seeds each were placed for each species under a fixed cyclic daily regime of light and temperature (10–25° C for 12 h each). The plates were monitored once a week for germination, and seeds that germinated and developed cotyledon or first leaves were counted. After 68 days, all seeds that did not germinate or moulded were cut to test their viability and estimate dormancy. Based on these tests, each seed was categorised into one of four categories: germinating, viable but not germinating (dormant), mouldy (die) or empty (see Table S1 in Supporting Information). Seed viability was determined for each species as the percentage of seeds classified as germinating or dormant.

RESULTS

Species richness was lowest in plots with positive correlation, intermediate in plots with no correlation and highest in plots of negative correlation ($F_{2,60} = 3.85$, $P = 0.03$, One-way ANOVA, Fig. 2a). Moreover, while communities with positive size/number correlation con-

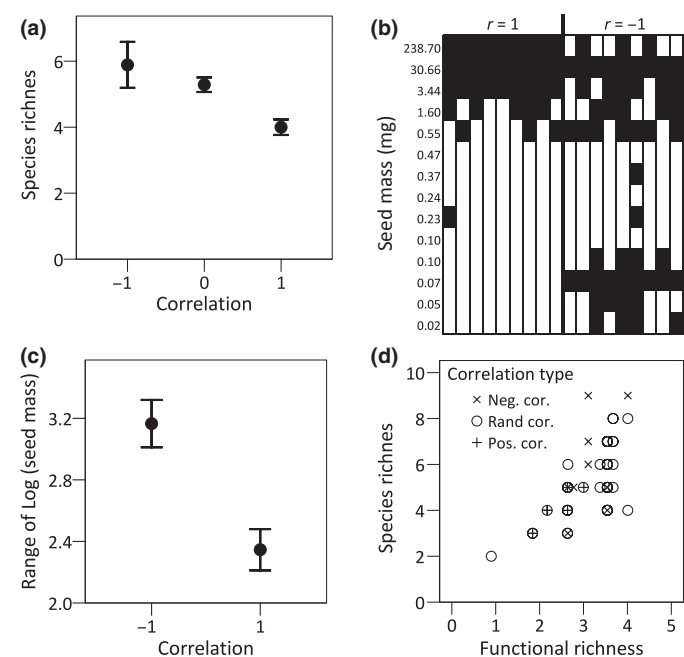


Figure 2 Effect of the seed size/number correlation on species richness (a), species composition (b), the range of among-species variation in seed size (c) and the relationship between species richness and log range of seed size (a measure of 'functional richness' sensu Vileger *et al.* 2008) (d). In all cases, error bars indicate standard errors. In (b), each column represents a community and each row represents a species with a certain seed size. Black/white cells indicate presence/absence, respectively, of the relevant species in the community. Note that small-seeded species are present only when their sowing density is high enough (i.e., under a negative correlation) and intermediate-seeded species do not attain a sufficiently high density under both correlations.

sisted almost entirely of large-seeded species, communities with negative correlation showed a mixture of large-seeded and small-seeded species (Fig. 2b), leading to a much larger range of seed size ($t = -4.016$, $P = 0.001$, Fig. 2c). As could be expected from these results, species richness was positively correlated with the range of seed size across communities (Fig. 2d). These overall results support the prediction that a seed size/number trade-off contributes to both species diversity and functional trait diversity by increasing the likelihood of small-seeded species to persist in the community.

A comparison of the empirically observed values of species richness in the community plots with their corresponding null-model predictions indicated that species richness was not significantly different from that expected at the absence of competition (5.19 vs. 5.20 species respectively, $t = 0.045$, d.f. = 124, $P = 0.964$). Moreover, our null models provided accurate predictions of both the frequency of occurrence and the number of species in the experimental communities (Fig. 3). Regression lines of observed vs. predicted values of both variables had intercepts not significantly different from zero, and slopes not significantly different from 1 (Fig. 3). These results indicate that the increase in species richness observed in communities with negative correlation between seed size and seed number cannot be attributed to a competition-colonisation trade-off.

Thus, our results confirm the hypothesis that a seed size/number trade-off is capable of promoting species richness, but cannot be related to a competitive advantage of large-seeded over small-seeded species and call for an alternative explanation. One such explanation might be non-competitive advantage of large-seeded species such as higher tolerance to environmental stress (Westoby *et al.* 1992; Dalling & Hubbell 2002; Muller-Landau 2010). Our demographic data allowed us to test two possible mechanisms that may provide such an advantage – higher density-independent seedling survival and higher probability of seedling emergence. Regression analyses showed that seed size did not affect seedling survival ($P = 0.486$), but had a significantly positive effect on emergence probability (Fig. 4). As can be expected, this effect led to a decrease in seedling density with the shift from positive to negative correlation between seed size and seed number ($r = 1 : 221.77 \pm 9.47$; $r = 0 : 89.13 \pm 7.52$; $r = -1 : 19.55 \pm 3.37$, $F = 45.507$; $P < 0.001$, one-way ANOVA). Although such a non-competitive mechanism may

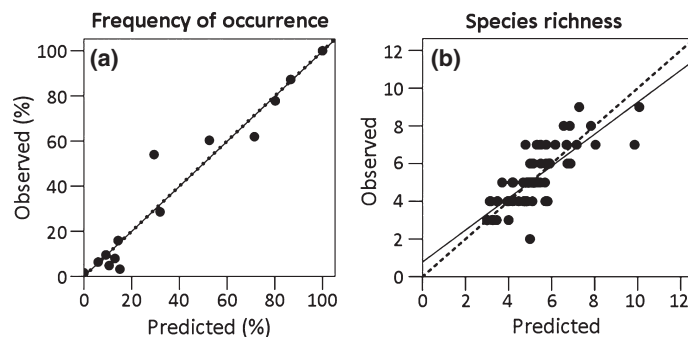


Figure 3 Relationship between observed frequencies of occurrence (a) and species richness (b) and the predictions of null models assuming no competition. In both cases, the linear regression (continuous line) does not differ significantly from the line $y = x$ (denoted by dashed line). Frequency of occurrence: $y = 0.998x - 0.000234$, $R^2 = 0.939$, $P < 0.001$; Species richness: $y = 0.848x + 0.781$, $R^2 = 0.636$, $P < 0.001$.

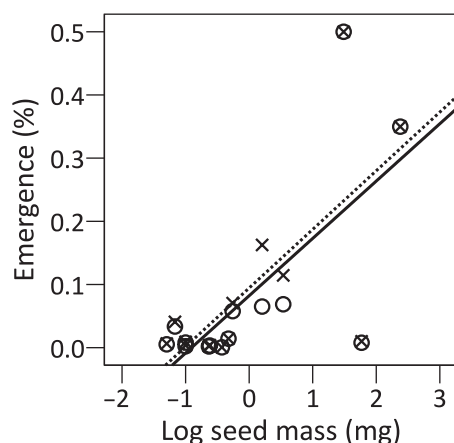


Figure 4 Effect of seed mass on the probability of emergence before (dots, continuous line) and after (crosses, dashed line) correction for among-species variation in seed viability. Uncorrected data: $R^2 = 0.73$, $P < 0.001$; corrected data: $R^2 = 0.77$, $P < 0.001$.

indirectly reduce competition intensity, our results indicate that differences in seedling densities among the experimental communities did not affect the proportion of species surviving to maturity ($R^2 = 0.001$, $P < 0.789$). We therefore conclude that higher emergence probability of large-seeded species, rather than higher competitive ability or higher seedling survival, was the main mechanism accounting for the effect of the seed size/number trade-off on species richness in our study system.

To further evaluate this conclusion, we tested the effects of seed size on germination probability and seed viability of the study species under controlled laboratory conditions. Results showed that seed size did not have any effect on germination probability ($R^2 < 0.01$, $P = 0.874$) or seed viability ($R^2 = 0.04$, $P = 0.874$), supporting our conclusion that the observed effect of seed size on emergence probability (Fig. 4) was caused by differences among species in tolerance to environmental conditions during the emergence stage. The germination tests also allowed us to determine the viability of the seeds used to establish the experimental communities, and thus, to correct for possible among-species differences in viability (i.e., to calculate emergence probabilities based on estimates of the number of *viable* seeds, rather than the total number of seeds sown). Such a correction increased the portion of variance explained by seed size from 0.73 to 0.77 (Fig. 4), further strengthening the conclusion that higher emergence probability was the main advantage of large seeds in our experiment.

DISCUSSION

Recently, there has been an increasing recognition that functional trade-offs and functional trait diversity (the extent of trait differences among species in a community, Tilman 2001) are key drivers of many ecological phenomena. This recognition has led to numerous studies on patterns, mechanisms and consequences of functional diversity in plant communities (Díaz *et al.* 2004; Westoby & Wright 2006). A fundamental assumption in many of these studies is that functional trade-offs contribute to both functional diversity and species diversity, but as far as we know, no study has directly tested this hypothesis. Here, we focus on the seed size/number trade-offs, one of the most widely studied trade-offs in plant communities, and demon-

strate that manipulations of this trade-off affect species diversity and functional diversity in a manner consistent with ecological theory.

Many previous studies have documented a negative correlation between seed size and seed number in natural communities (Rees 1995; Eriksson & Jakobsson 1998; Turnbull *et al.* 1999; Jakobsson & Eriksson 2000, 2003; Levine & Rees 2002). Such correlations have usually been interpreted as a support for the hypothesis that a seed size/number trade-off promotes coexistence in plant communities. However, taking into account the wide range of stabilising and equalising mechanisms of coexistence that operate in plant communities (Amarasekare 2003; Adler *et al.* 2007; Turnbull *et al.* 2008), an observed negative correlation between seed size and seed number by itself does not imply that the underlying coexistence mechanism is a seed size/number trade-off.

To directly demonstrate the effect of such a trade-off one needs to experimentally manipulate the correlation between seed size and seed number and show that positive correlation reduces, while negative correlation promotes the number of species in the community. In this study, we employed such an approach in a system of annual plants. By manipulating the correlation between seed size and seed number in experimental communities, we were able to demonstrate that (1) a negative correlation increases while a positive correlation reduces the number of species in a community (Fig. 2a), (2) a negative correlation between seed size and seed number increases the magnitude of among-species variation in seed size (Fig. 2b and c) and (3) species richness and the range of seed size in a community (a measure of functional richness) are positively correlated (Fig. 2d). These findings support the hypothesis that a seed size/number trade-off may increase the number of species in a community by allowing coexistence of large-seeded and small-seeded species, thereby promoting functional diversity. This mechanism is expected to be particularly important in annual species where much of the dynamics is determined by seed traits (Levine & Rees 2002).

Although these results are consistent with theoretical expectations, two main limitations of our study should be recognised. First, microcosm experiments such as those performed in this study do not represent real environments. Consequently, demonstrating the operation of a particular mechanism in such experiments does not imply that the relevant mechanism is important under natural conditions. Second, results obtained over a short time scale are not necessarily applicable to longer scales. Specifically, the fact that a seed size/number trade-off increased species richness over a single growing season in our experiment does not imply that it is capable of maintaining a long-term coexistence. Thus, although we used a collection of species that co-occur in natural habitats, this study should be regarded as an experimental test of theoretical hypotheses under artificial conditions, rather than a demonstration of mechanisms operating in natural communities of these species.

The overall results allow testing a number of previously proposed hypotheses concerning the mechanisms by which large-seeded species maintain viable populations in spite of a lower fecundity. Although most authors agree that the advantage of large seeds should be expressed at the seedling stage, the actual mechanisms by which a large seed size compensates for a small seed number are under a continuous debate (Westoby *et al.* 1992; Leishman 2001; Coomes & Grubb 2003; Turnbull *et al.* 2005, 2008; Muller-Landau 2010). The original and most frequently invoked hypothesis is a trade-off between competitive ability and colonisation ability (i.e., a competition-colonisation trade-off, Rees 1995; Geritz *et al.*

1999; Jakobsson & Eriksson 2000; Leishman 2001). According to this hypothesis, a lower colonisation ability of large-seeded species due to their smaller seed number is balanced by superiority in competitive interactions at the seedling stage. However, theoretical studies demonstrate that this mechanism can only be effective if competitive interactions are extremely asymmetric (Geritz 1995; Adler & Mosquera 2000; Levine & Rees 2002). Other requirements of this mechanism such as the positive correlation between seed size and competitive ability and the better colonisation ability of small-seeded species have also been questioned (Leishman 2001; Jakobsson & Eriksson 2003; Eriksson 2005; Turnbull *et al.* 2005). Furthermore, Moles & Westoby (2004b) compiled data on seedling densities from published literature and concluded that natural populations of seedlings seldom occur at high enough densities for seedling–seedling competition to be a major cause of mortality (but note that competition may also influence reproduction rates, see Rees & Westoby 1997).

In this study, we explicitly tested the competition-colonisation trade-off hypothesis by comparing the number and frequency of occurrence of species in experimental communities with corresponding predictions based on null models assuming no competition. Null models gave accurate predictions of both species richness and frequency of occurrence, indicating that competitive interactions were not important in determining these variables. While competitive interactions may have influenced other plant attributes (e.g., plant biomass or seed production), these findings demonstrate that our results cannot be attributed to a competition–colonisation trade-off.

Levine & Rees (2002) proposed an alternative mechanism of seed size/number trade-off in which large-seeded species are better competitors and small-seeded species compensate for their competitive disadvantage by being more tolerant to a wider range of environmental conditions. According to this ‘included niche model’ (following the terminology of Colwell & Fuentes 1975), small-seeded species should show higher rates of germination and/or emergence than large-seeded species at the absence of competition due to their tolerance of a wider range of environmental conditions. Our results show an opposite pattern, thus allowing us to reject this mechanism.

The results of this study best fit the establishment-colonisation trade-off proposed by Dalling & Hubbell (2002). They analysed patterns of seedling establishment in a system of neotropical pioneer species where seed mass varies over four orders of magnitude and concluded that this variation was maintained by a trade-off between establishment and colonisation abilities. They further argued that this trade-off is not necessarily a competition–colonisation trade-off, as seed densities were too low for direct seedling competition to occur. The observed effect of seed size on seedling emergence in our study (Fig. 4), together with the lack of evidence for the effect of competitive interactions on species richness and frequency of occurrence (Fig. 3) are consistent with such an establishment–colonisation trade-off.

The hypothesis that a seed size/number trade-off may promote species richness at the absence of competition receives theoretical support from a recent model that trades-off stress tolerance and fecundity (Muller-Landau 2010). In this analytical model, large-seeded species better tolerate environmental stress at the establishment stage, but produce a smaller number of seeds, whereas small-seeded species have low stress tolerance at the establishment stage but higher fecundity (Muller-Landau 2010). Consistent with our findings, this model demonstrates that a trade-off between seed

size and seed number may contribute to species richness without any need to incorporate direct competitive interactions.

Unfortunately, our study is not directly comparable with other empirical studies. The closest study is a sowing experiment conducted by Turnbull *et al.* (2005) in a system of short-lived grassland species. This experiment was designed to discriminate between the predictions of the three mechanisms of trade-off discussed above (competition–colonisation, included niche, and establishment-colonisation). As in our experiment, seed mass had a positive effect on seedling density, but no effect on seedling survival. Related sowing treatments focusing on interspecific interactions revealed no evidence for competitive exclusion. Thus, competition–colonisation trade-off was not important in determining species diversity in that system and the overall findings were most consistent with the establishment–colonisation model, as in our experiment.

These experimental results are highly relevant for the ongoing discussion concerning the role of deterministic vs. stochastic determinants of community assembly (Gravel *et al.* 2011). Deterministic theories emphasise the interaction between species’ life history traits and the environment, while stochastic theories emphasise the role of propagule supply as the main determinants of community structure (Hubbell 2001). The fact that both seed size and seed number were important in determining the establishment of individual species and the number of species in our experimental communities supports recent theoretical (Gravel *et al.* 2006; Kadmon & Allouche 2007) and empirical (Ellwood *et al.* 2009; Foster *et al.* 2011; Myers & Harms 2011) evidence, showing that both kinds of processes may operate simultaneously in determining the diversity of ecological communities.

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AUTHORSHIP

EBH and RK designed the study, AS conducted the laboratory germination tests, OFS, RH and AS provided botanical methods and materials, EBH performed all parts of the experiment, analysed the data and wrote the first draft of the manuscript and RK revised the manuscript.

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