Downsized mutualisms: consequences of seed dispersers body-size reduction for early plant recruitment

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Summary

Extinction-driven, body-size reduction of seed dispersers (i.e., an ecological downsize resulting from severe defaunation) can entail the loss of unique ecological functions, and impair plant regeneration. However, how the downsize of mutualistic animals affects different scales of seed dispersal and plant recruitment remains understudied. Here, we took advantage of a natural experiment in the Canarian archipelago to document the consequences of body-size reduction of the lizards (*Gallotia*, Lacertidae*)* on recruitment of *Neochamaelea pulverulenta* (Rutaceae), which relies exclusively on these frugivores for seed dispersal. Subsequent to the arrival of humans (ca. 2000-2500 yr BP), the extinction of large-bodied lizards generated a gradient of increasing defaunation in the three islands inhabited by this plant. We hypothesized a significant reduction, and eventually collapse, of the early seedling recruitment mirroring the defaunation intensity of the frugivores. We sampled 42 populations spanning the whole geographic range of the plant to examine the quantitative (age structure pattern) and the qualitative components of plant regeneration: proportion of seedlings growing outside the canopy, number of seedlings established outside canopy relative to the number of adults (effective recruitment rate), and seedling vigour. The age structure distributions did not differ among the three contrasted ecological scenarios. However we found significant reductions in seedling recruitment outside the canopy, effective recruitment rate, and delayed negative effects on seedling vigour in populations hosting small- to medium-sized lizard species. Extirpation of large seed-dispersers did not cause substantial reductions in quantitative components of seed dispersal, but determined declines in qualitative aspects critically affecting dispersal effectiveness. Our study highlights the importance of examining all components of the dispersal/recruitment process to properly document the regeneration outcomes of plants in defaunated, downsized ecological scenarios.

Keywords: Canary Islands, defaunation, *Gallotia,* lizard extinction, *Neochamaelea pulverulenta*, seed dispersal.

Introduction

Extinction of vertebrate species has been a recurrent, but not taxonomically random, pattern throughout the Earth's history (Raup, 1986; Shodhi et al., 2009). For example, mass extinction events have frequently unfolded dramatic reduction of the number of large-bodied species, as occurred after the disappearance of dinosaurs in the Cretaceous-Tertiary transition (Sheehan et al., 1991), the megafauna in the Late Pleistocene (Alroy, 2001), and also the present-day defaunation (Barnosky et al., 2011). This phenomenon often results in transitions from pristine communities, where large species are relatively abundant, to downsized communities dominated by small- to medium-bodied vertebrate species (Peres and Dolman, 2000), a pattern of ecological downgrading entailing the loss of unique ecological functions (Estes et al., 2011; Dirzo et al., 2014). Since the ecological roles of large species are disproportionately important in ecosystem dynamics (Cordeiro and Howe, 2001, 2003; Woodward et al., 2005; Wright et al., 2007; Johnson, 2009), the effect of their extinction is expected to cascade through the remainder of the biota and produce deep shifts in composition, structure and function of downsized communities (Redford and Feinsinger, 2001; Rule et al., 2012; Harrison et al., 2013). A critical issue is thus to develop research frameworks potentially enabling a better forecasting of cascading effects and the potential for delayed consequences of extinction-driven body size reduction and the deterioration of their associated ecological functions (Dirzo et al., 2014).

Animal-mediated dispersal is a crucial process in the life cycle of many flowering plants. It allows seeds and seedlings to escape the higher mortality frequently associated to the adult neighbourhood (Janzen, 1970), colonize new sites (Howe, 1982) and promote gene flow within and among populations (Hamrick et al., 1993). Large frugivores have an important role in all of these components of seed dispersal because they can consume a higher amount of fleshy fruits, disperse larger seeds and move them to longer distances than the smaller species in the mutualistic assemblages (Jordano et al., 2007; Muller-Landau, 2007; Wotton and Kelly, 2011). Thus, there are a numerous ways in which natural regeneration, especially of large seeded plants, can be impaired by a drastic body size reduction in the frugivore assemblages. For example, if frugivores become smaller, plants bearing large fruits can have strong limitations to disperse seeds because frugivore gape width constrains the maximum fruit size animals can successfully handle and swallow (Wheelwright, 1985). Late-acting, post-dispersal effects may unfold, preventing or severely limiting seedling recruitment, and leaving defaunated ecosystems full of living-dead adult plants (Janzen, 1986) or with highly clumped regeneration within the neighbourhood of parent plants (Cordeiro and Howe, 2001). In addition, the extinction of large frugivores may even trigger rapid evolutionary responses, given that the extant small frugivores promote selection for reduced seed-size (Galetti et al., 2013). Reduction of seed size may in turn negatively impact plant recruitment since seed size is positively correlated with seedling size, growth rate and seed reserves which increase seedling survival under stress conditions (Howe and Richter, 1982; Moles and Westoby, 2004). Therefore, the downsizing of mutualistic frugivores can affect multiple scales of their interaction with plants, yet most of these aspects remain largely unknown.

The effects of large frugivore declines are expected to be much more pervasive on species-poor ecosystems such as oceanic islands. On one hand, extinction or body-size reduction of frugivore species has been dramatic on islands (Hansen and Galetti, 2009) and quite often preceded by the extinction of their functional roles (McConkey and Drake, 2006; Boyer and Jetz, 2014). On the other hand, insular environments frequently present low functional redundancy of dispersal agents (e.g. Woodward et al., 2005; Wotton and Kelly, 2011). Thus, seed dispersal may collapse in defaunated insular scenarios, causing substantial reductions of plant recruitment. Previous studies have been addressed the demographic consequences for plants when seed dispersal disruption occurs (Meehan et al., 2002; Traveset and Riera, 2005; Rodríguez-Pérez and Traveset, 2009; Wotton and Kelly, 2011, 2012; Traveset et al., 2012). However, as far as we know, none of them have tracked the demographic consequences through a progressive deterioration of the dispersal services as a result of downsizing of interacting animal species.

Lizard-mediated seed dispersal has been described as a widespread mutualism on oceanic islands (Olesen and Valido, 2003; Valido and Olesen, 2007). In the Canary Islands, endemic lacertid lizards (g. *Gallotia*) are extremely important seed dispersers in all islands and habitats (Valido and Nogales, 1994; Valido, 1999; Valido and Nogales, 2003; Valido et al., 2003; Rodríguez et al., 2008). However, the arrival of humans (ca. 2000-2500 yr BP) triggered a process of lizard species extinction and body size reduction in these islands (Barahona et al., 2000). The pattern and magnitude of these extinction processes has been markedly different in each island, with defaunation-mediated lizard downsizing ranging from subtle (Gran Canaria) to noticeable (Tenerife), to very marked (La Gomera).

Here, we document the effects of body size reduction of Canarian lizards on the early seedling recruitment of *Neochamaelea pulverulenta*, an endemic large-seeded treelet dispersed exclusively by medium- to large-sized frugivorous lizards (Valido, 1999). Our approach is a comparative analysis among the three islands where *N. pulverulenta* is distributed, which define a gradient of extinction-driven lizard body size reduction: Gran Canaria preserves the largest extant lizard species, *G. stehlini*; Tenerife has abundant medium-sized *G. galloti* lizards, whereas La Gomera hosts the smallest-sized species *G. caesaris* (see Appendix 1, Fig. S1 and S2 in the supplementary data for further details). We hypothesize that since larger lizards consume more and larger fruits, the extinction-driven body size reduction will negatively affect both quantitative and qualitative components of *N. pulverulenta* recruitment causing: *i*) differences among islands in overall recruitment patterns (age structure), *ii*) a decrease in the proportion of seedlings recruiting outside the canopy of adult plants, *iii*) a reduction of the effective recruitment rate of seedlings (per capita of adult plants), and *iv*) a reduced vigour of seedlings, resulting from the collapse of consumption of large-fruits (with large seeds).

Material and methods

Study Species

Neochamaelea pulverulenta (Rutaceae) (Vent) Erdtman is an endemic treelet from the Canary Islands distributed in the lowland (< 400 m a.s.l.) xerophytic areas from Gran Canaria, Tenerife and La Gomera. In these zones the average annual temperature and precipitation are around 21°C and 200 mm, respectively (AEMET and IP, 2012). This lowland vegetation is also composed by different species of *Euphorbia* (Euphorbiaceae), *Rubia fruticosa*, *Plocama pendula* (Rubiaceae), *Periploca laevigata* (Asclepiadaceae), *Lavandula* spp. (Labiatae), *Lycium intricatum* (Solanaceae), among others. Adult plants of *N. pulverulenta* average 1.1 m height but some individuals can reach 2.7 m (n = 2132). Plants can bloom almost throughout the year, but usually twice, on winter and spring periods. Their flowers are pollinated mainly by ants, solitary bees, and flies. Fruits include 1 to 4 cocci (11.1 ± 1.6 mm in diameter each; Valido, 1999). The coccus can be considered functionally a drupe composed by fleshy pulp containing invariably one hard-coated seed (8.6 ± 1.0 mm in diameter; Valido, 1999).

Only medium- and large-bodied lizards (g. *Gallotia,* Lacertidae) eat these fruits, adequately handling and swallowing individual cocci, acting also as legitimate seed dispersers (Valido and Nogales, 1994; Valido, 1999; Valido et al., 2003). Secondary dispersal by raptor predators on lizards containing seeds of *N. pulverulenta* has been also documented (Padilla et al., 2012). Before human colonization (ca. 2000-2500 yr BP, Appendix S1), giant lizard species were widespread in the western and central islands (Barahona et al., 2000). Subsequently, different extinction scenarios emerged in each island resulting in a marked downsizing of the extant lizard species. However, the intensity and consequences of this process were different in the three islands where *N. pulverulenta* is present, allowing us to define three different ecological scenarios along a gradient of lizard body-size variation (Fig. S2). In Gran Canaria lizard downsizing has been relatively minor, from the extinct *G.* aff. *stehlini* (maximum snout-vent length, max SVL = 367 mm) to the extant *G. stehlini* (max SVL = 280 mm). In contrast, lizard size reduction has been very intense in La Gomera, where the actual widespread species (*G. caesaris*, max SVL = 111 mm) is 4-fold smaller than their extinct relatives (*G. goliath,* max SVL = 466 mm). In turn, in Tenerife *G. goliath* (max SVL = 502 mm) become extinct and currently only the medium-sized species *G. galloti* (max SVL = 145 mm) is widely distributed. Moreover, large species *G. intermedia* (max SVL = 174 mm) and *G. bravoana* (max SVL = 212 mm) are present in Tenerife and La Gomera respectively, but they are critically endangered, surviving only in extremely reduced populations on highly localized, inaccessible cliffs (Figs S1and S2 and references therein for details).

Plant demography

To carry out the comparative study of plant recruitment under different seed dispersal scenarios, we sampled 42 populations of *N. pulverulenta* from three islands: Gran Canaria (*n* = 11), Tenerife (*n* = 19), and La Gomera (*n* = 12). These populations adequately span the whole insular distribution and the range of climatic conditions of plant populations (Table S1, Fig. S3). In each population we set up 3-6 linear transects (25-100 m length; 5 m wide) depending on plant population size. Along these transects we measured all individual plants, except for seedlings. Because seedlings were very abundant in some populations, we haphazardly measured a subset of them (mean: 29.3 %, *n* = 637 measured individuals). The individual plant measures include the basal trunk/stem diameter (using a digital caliper), the maximum height, and the two major diameters of the vertical canopy projection (using a measuring tape). Moreover, each individual plant was categorized (including non-measured individuals) according to age class (seedling, sapling, juvenile, adult). Seedlings were identified as plants < 1 mm of stem diameter and with less than four leaves. Individuals not branched, < 7mm stem and not recorded as seedlings were labelled as saplings. Plants between 7-15 mm basal trunk diameter and no evidence of reproduction (absence of floral buds and/or seeds beneath the plant) were recorded as juveniles. On average we collected data from 228 plants per population (range: 102-571), with a total of 9402 plants sampled. Then, we used this dataset to obtain the age structure pattern (the frequency distribution of each age class) in the sampled populations.

For a subset of 32 populations (Table S1, Fig. S3), we also kept information on the proportion of seedlings established outside the canopy of *N. pulverulenta* plants and the effective recruitment rate of seedlings along the transects. For the first variable, we recorded the number of seedlings located > 1m away from the canopy of the nearby adult plant relative to the total seedlings recruited. We also estimated the effective recruitment rate of seedlings (number of seedlings outside the canopy relative to the number of adult plants). This demographic parameter indicates the per-adult number of seedlings successfully recruiting away from adults (outside the canopy), i.e., recruiting from effectively dispersed seeds.

Finally, for the analyses of seedling vigour (i.e. stem diameter) we selected 22 populations with at least 6 seedlings measured (range: 6-93 seedlings, depending on seedling abundance; Table S1).

Lizard abundance and other plant population parameters

We obtained an index of relative abundance of medium- to large-bodied lizards in the 32 populations previously selected for the detailed analyses of seedling recruitment. Given that lizard body-size and diameter of the droppings are positively correlated (Valido and Nogales, 2003), we recorded the number of medium- to large-sized droppings (> 4 mm in diameter) on 50 quadrats (0.5 x 0.5 m), regularly spaced 5 m apart along each of 5 linear transects (spaced 10 m) per population.

To obtain an estimate of plant density per population we set up two perpendicular 100 x 4 m transects, where we counted all the *N. pulverulenta* adult plants rooted within the transect.

These data are independent of the adult plants censused in the age structure sampling. In addition, we gathered climatic data from meteorological stations located up to 7 km distance (1 to 4 stations), with long temporal data series available (range = 6 – 53 years). For each population we recorded the average annual precipitation (pp), the mean maximum temperature of the hottest month (t_{max}), and the mean minimum temperature of the coldest month (t_{min}). Then, we calculated the Emberger index (Emberger, 1955) defined as $Q = (2000\, pp)/(t_{max}^2 - t_{min}^2)$ and log-transformed it ($-LnQ$; Tieleman et al., 2003) for statistical analyses.

Statistical Analyses

To examine differences in the age structure pattern (relative frequencies of each age class) among islands we fitted two generalized linear models (GLM) with a binomial distribution of the errors, a log link function and "island" as a fixed factor using data from all sampled populations ($n = 42$). In the first model we tested for island differences in the proportion of subadults (pooled number of seedlings, saplings, and juveniles) versus the proportion of adult plants and, in the second model, we tested for differences in the proportion of seedlings relative to the rest of pooled age classes (saplings, juveniles, adults).

To assess the effect of the ecological scenarios (islands) on the proportion of seedlings outside the canopy we applied GLMs with a binomial distribution of errors and a logit link function. We used "island" as the main fixed factor and both density of *N. pulverulenta* adult plants and Emberger index as covariates.

Among-island differences in the effective recruitment rate of seedlings were tested by fitting a GLM with a Poisson distribution of the errors and a log link function. The number of seedlings was used as a response variable, while the "island" was used as a fixed factor, Emberger index as a covariate and the number of adults per population as an offset of the model.

The stem diameter of sampled seedlings was used as an estimate of seedling size and vigour. We tested variation in seedling vigour among islands by fitting a linear mixed model (LMM) using “island” as the main fixed factor with population identity as a random factor nested within it and the Emberger index as a covariate.

In order to assess differences in all measured demographic parameters among pairs of islands we used post-hoc contrasts (Tukey test). In addition, we checked for spatial autocorrelation among model residuals by performing a multivariate Mantel correlograms. Given that spatial autocorrelation was not detected for any of the above demographic parameters, we did not include spatial information in the previous models (details on Appendix S2, Fig. S4).

Finally, we tested for island effects on density of medium- large droppings, as a proxy of relative density of large- to medium-sized lizards. We used an ANOVA procedure with post-hoc contrasts (Tukey test) to test for differences among islands. All the statistical analyses were carried out with R packages (R Development Core Team, 2014).

Results

The age structure pattern of *N. pulverulenta* was highly variable among populations within each island, ranging from aged populations, where most individuals were adults (e.g. Montaña de Tabaiba, Gran Canaria), to relatively younger stands where many censused plants were subadults (e.g. Barranco de la Negra, La Gomera) (Table S1). When considering the relative frequencies of subadults relative to adult plants, differences were not detected despite marked differences in the three insular scenarios (GLM, p-value > 0.05; Fig. S5). In turn, the average percentage of seedlings was consistently similar among islands, varying from 31.0 ± 27.8 % in Tenerife to 25.1 ± 20.7 % in Gran Canaria (GLM, p-value > 0.05; Fig. S5).

The proportion of seedlings outside the canopy was significantly different among islands: Gran Canaria (12,7 %), Tenerife (17,7 %) and La Gomera (2,8 %) (Table 1, Fig. 1). In the model, the effect of *N. pulverulenta* adult plants density was statistically significant ($Z = -3.19$, p-value <

0.001). Moreover, climatic conditions did not influence differences on this demographic parameter.

Regarding the effective recruitment rate of seedlings, we detected significant differences among all pairwise comparisons (Table 1, Fig. 2a). Populations in La Gomera recruit significantly fewer seedlings per adult (0.01 seedlings/adult) than those in Gran Canaria (0.06 seedlings/adult) and Tenerife (0.39 seedlings/adult). In this case, only Emberger index seems to be significant (Z = 6.05, p-value < 0.001), indicating higher effective recruitment in more arid populations.

The observed differences among islands in the proportion of seedlings outside the canopy and the effective recruitment rate of seedlings match the variability detected in the abundance of medium to large lizard droppings. In the particular case of La Gomera, large droppings were totally absent in the sampled populations (Fig. 2b). Also, we recorded a significantly lower density of lizard droppings in Gran Canaria than in Tenerife (t-value = 2.58, p-value < 0.05).

Finally, we found consistently thinner seedlings (stem diameter) in Tenerife, with no differences between Gran Canaria and La Gomera (Fig. 3, Table 1). Variation in stem diameter across populations did not show any association with the Emberger index.

Discussion

By using a natural island-based field experiment from the Canary Islands we found that defaunation-mediated downsizing of frugivorous lizards critically hampers some components of the early recruitment of *N. pulverulenta*. Unexpectedly, our results suggest that reduction of lizard body size had not effects on the quantitative component of seed dispersal (i.e. the relative abundance of seedlings) in the different insular scenarios. However, we detected critical effects on the qualitative components, such as a reduction of seedling establishment away from adult plants, and delayed negative effects on seedling vigour. First, there was a marked collapse of recruitment beyond the neighbourhood of adult plants in the ecological scenario (La Gomera) where a drastic reduction of lizard body-size has occurred. Second, the reduced seedling stem diameter observed in populations hosting medium-sized lizards (Tenerife) suggests that even a relative small decline of lizard body-size may result in less vigorous seedlings when compared with the scenario hosting giant lizards (Gran Canaria). These differences are not attributable to variation in climatic factors or soil characteristics, but appear closely associated to the downsizing pattern. Our results broadly support the patterns reported in previous studies where plant-frugivore mutualism disruption affected the quantity and/or the quality of plant regeneration (e.g. Chapman and Chapman, 1995; Cordeiro and Howe, 2003; Traveset and Riera, 2005; Galetti et al., 2013). Yet our study highlights that situations with reduced or collapsed dispersal services can remain undocumented if not all components of dispersal effectiveness are studied, as indicated by the significant reductions in both effective dispersal and seedling vigour in the downsized scenarios.

Quantitative consequences for plant demography

We found a similar proportion of seedlings despite the marked differences in lizard body-sizes among islands. In fact, we found a relatively high frequency of established seedlings (> 25 %) in most populations. These populations do not differ in soil type (volcanic substrate) or climatic conditions (i.e. aridity; Table S1), so the similarity of the inter-insular demographic pattern can not be attributed to compensatory effects of abiotic conditions (e.g. favourable conditions for establishment in areas with limited dispersal by lizards). Biotic interactions could have also compensatory effects associated, for instance, if differences in herbivory and/or post-dispersal seed predation intensities counterbalance the effect of dispersers, yet we have no results supporting this (e.g. we have no records of herbivory on seedlings). The lack of differences in overall recruitment contrasts with previous studies indicating reductions of recruit density in systems hosting non-effective seed dispersers (e.g. Cordeiro and Howe, 2003; Traveset and Riera, 2005, but see Bleher and Böhning-Gaese, 2001 for similar results).

In our study system, several factors might explain the large proportion of seedlings of *N. pulverulenta* observed in the three islands. A very large fraction of the fully-developed fruit crop falls beneath parents, usually during early summer. Thus, it is frequent to find large amounts of *N. pulverulenta* seeds without pulp beneath conspecific plants. Small lizards can bite and tear-off the pulp without removing the fruit, thus not acting as legitimate dispersers but potentially enabling seed germination (Fig. S6). In addition, we have evidences that rodents consume the pulp and leave seeds accumulating beneath maternal plants (personal observation). Besides, these animals can probably move a minor proportion of these seeds away from mother plants. Thus, seed movement by runoff and facilitation of germination by secondary frugivores and/or post-dispersal seed predators is most likely contributing early establishment even in situations with limited or absent legitimate dispersal (La Gomera).

Qualitative consequences for plant demography

Despite the absence of differences in the age structure pattern among islands, a clear inter-insular pattern emerges when considering several qualitative components of seed dispersal effectiveness (Schupp et al., 2010), i.e. proportion of seedlings outside the canopy of adult plants, effective recruitment rate of seedlings, and seedling vigour.

At one extreme of the defaunation and downsize gradient (La Gomera), we found the lowest values on both the proportions of seedlings outside the canopy and the effective recruitment rate of seedlings. These results, together with an absolute absence of seeds of *N. pulverulenta* in the Gomeran lizard droppings, indicate the collapse of the lizard-mediated dispersal interactions in this island. This contrasts with data recorded from islands hosting medium- and large-bodied lizards (Tenerife and Gran Canaria, respectively). The human-driven extinction of the known largest species in La Gomera (*G. goliath*) and the marginal presence of the extant giant lizard *G. bravoana* only in a remnant isolated population (Valle Gran Rey), have actually deprived *N.*

pulverulenta of effective seed dispersers throughout the island. The remaining abundant species *G. caesaris* is unable to effectively handle fruits and seeds due to marked morphological restrictions (i.e. fruit size considerably exceeds gape width of the lizard; Valido, 1999), a factor potentially impairing fruit removal and effective seed dispersal. Significant reductions of seeds dispersed away from adult parents have been reported in other defaunated scenarios (Chapman and Chapman, 1995; Cordeiro and Howe, 2003) where the lack of efficient dispersal agents collapses dispersal in distance and most seeds accumulate beneath maternal canopies.

The plant populations from La Gomera, however, still preserve a marginal effective recruitment. We recorded approximately 3% of recruited seedlings established beyond the vicinity of adult plants and a very low (but non-zero) effective recruitment rate. Small-sized *G. caesaris* that frequently steal the fleshy pulp from fruits of the undispersed crop, can sporadically move away some fruits and remove the pulp away from adult plants where a minor fraction of seeds can likely germinate. In addition, *N. pulverulenta* populations usually appear distributed on ravine slopes, where these seeds without pulp can be dispersed by rain or gravity. Lastly, although rodents mostly act as seed predators they can also disperse some seeds infrequently. Similar results have been reported for other plants (Traveset and Riera, 2005; Guimarães et al., 2008) where vertebrate-mediated seed dispersal has been disrupted and plants rely just on haphazard, marginal dispersal. In fact, a recent study tracking seed fates (Jansen et al., 2012) showed that scatter-hoarding rodents provide effective seed dispersal to widowed plants, acting as substitutes of an extinct megafauna.

We would expect the largest fraction of seeds being dispersed away from maternal plants in Gran Canaria, whose populations currently host the largest-sized lizards (*G. stehlini*). However, contrary to this expectation, the probability of finding seedlings recruiting beyond the parent plants and the effective recruitment rate of seedlings was higher in the island (Tenerife) hosting medium-sized lizards (*G. galloti*). Neither abiotic factors nor differential enemy-mediated mortality of seeds/seedlings, as discussed above, help explaining this inter-insular pattern. A more plausible explanation relates to the variation in abundance of Canarian lizards in both islands. It is known that larger lizards are relatively less abundant than small ones (Buckley and Jetz, 2007). At this respect, our estimates indicate that *G. galloti* in Tenerife is 6-fold more abundant than *G. stehlini* in Gran Canaria. This result suggests that increased abundance of medium-sized lizards in Tenerife could explain the large number of seedlings found beyond the maternal plants, i.e., a type of compensatory mass effect directly favouring higher fruit removal rates and dispersal. This supports the idea that the contribution of low-effective animal mutualists to reproductive success of plants may be frequently overcompensated by their abundance (Vázquez et al., 2005).

A key variable driving the outcome of mutualistic interactions with gape-limited frugivores is seed size (Wheelwright, 1985). Small-bodied/gaped frugivores do not adequately handle and process large fruits or seeds, effectively truncating the seed size range in the seed rain. Large bodied-frugivores usually disperse larger seeds and a wider range of seeds sizes, so potentially favouring large seeds (Valido, 1999; Galetti et al., 2013) which result in larger seedlings (Howe and Richter, 1982; Moles and Westoby, 2004). We hypothesized that body-size reduction of mutualistic lizards could entail a late-acting reduction of seedling vigour of *N. pulverulenta* due to consistent size reductions of successfully removed seeds. Our results partially support this hypothesis. On one hand, we found a consistent and significant reduction of seedling stem diameter in populations hosting medium-sized lizards (Tenerife) when compared to populations with large-sized lizards (Gran Canaria). On the other hand, seedlings in La Gomera were unexpectedly thicker than seedlings in Tenerife, and similar to those in Gran Canaria. As previously discussed, these differences do not relate to climatic conditions or soil type differences. Thus, the large seed sizes and vigorous seedlings observed nowadays in La Gomera would reflect the phenotypic selection pattern on fruit size exerted by giant lizard in the recent past (Valido, 1999) and the more recent extinction events.

In summary, our comparative approach included three well-constrasted ecological scenarios along a gradient of progressive reduction of the frugivores body sizes. After controlling for variation in abiotic conditions, differences in the early recruitment of a plant species with exclusive dependence on medium- to large-bodied lizards for seed dispersal mirrored this defaunation-mediated downsizing gradient. At one extreme, Gran Canaria populations illustrate a scenario of preserved interactions, Tenerife an intermediate suboptimal scenario, whereas La Gomera exemplifies a scenario where both the seed dispersal process and the regeneration away from maternal plants have collapsed. The example of La Gomera is paradigmatic since plant populations have persisted over 500 years without their effective seed disperser partners, as reported for other widowed megafaunal-dispersed plants surviving more than 10,000 years (Janzen and Martin, 1982; Guimarães et al., 2008). Reliance on secondary dispersal has been proposed as a key mechanism underlying this persistence of widowed plants (Guimarães et al., 2008; Jansen et al., 2012). Accordingly, our results suggest that in the case of *N. pulverulenta* the very limited secondary dispersal mediated by abiotic and biotic vectors in combination with an apparently low seedling mortality under parent plants could be allowing the long-term local persistence of the plant populations in La Gomera.

Given that anthropogenic impact is causing a very fast decline of frugivore size on islands, where the projected downsizing in the future is up to three orders of magnitude above mainland ecosystems (Hansen and Galetti, 2009), it is likely that our results can be broadly extrapolated. Extirpation of large-bodied frugivores may not cause dramatic declines in some quantitative components of dispersal (Markl et al., 2012), but will certainly determine reductions in qualitative aspects critical to ensure dispersal effectiveness. It remains to be seen if this downsizing pattern also drives reduced gene flow via seed dispersal within and among populations. Moreover, it would be interesting to assess to what extent these patterns of reduced gene flow will have a lasting signal in the genetic structure of plant populations both at local and regional scales.

Meanwhile, our results highlight the importance of conserving the full range of functional processes (qualitative and quantitative components) involved in mutualistic interactions to assure the persistence of local regeneration and plant population dynamics in a changing world.

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Appendix A. Supplementary data

The following are the supplementary data to this article:

Appendix S1. Natural history of the Canarian lizards (g. *Gallotia*, Lacertidae).

Appendix S2. Additional information for the statistical analyses.

Figure S1. Distribution of *Gallotia* species in the Canary Islands.

Figure S2. Downsizing patterns of *Gallotia* species in the islands where *N. pulverulenta* (Rutaceae) is present.

Figure S3. Distribution map of sampled *N. pulverulenta* populations.

Figure S4. Autocorrelograms of the models residuals.

Figure S5. Age structure patterns at the island scale.

Figure S6. Photo-collage illustrating the study system.

Table S1. Information about the sampled *N. pulverulenta* populations.

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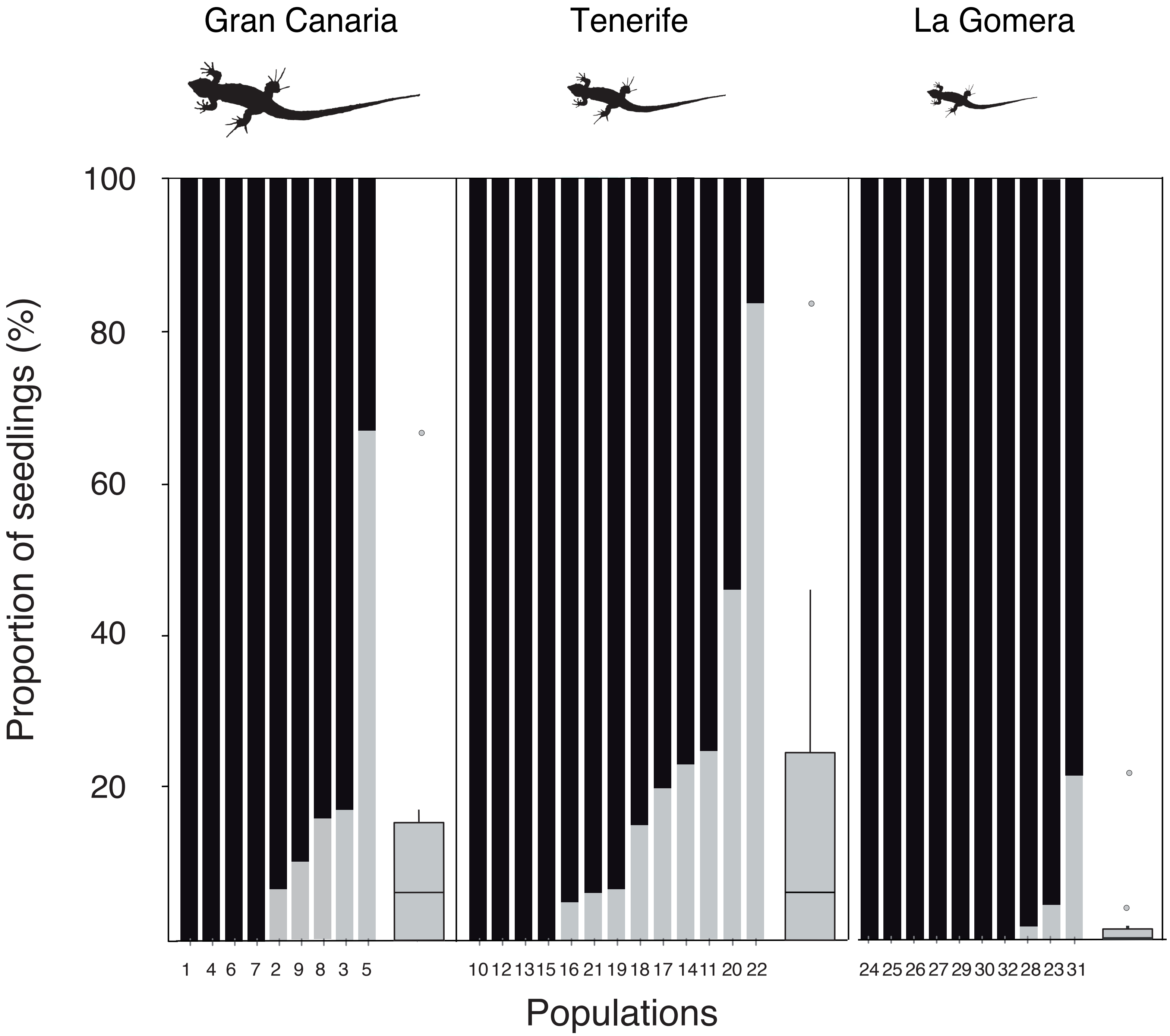
Fig. 1. Proportion of *N. pulverulenta* seedlings growing outside (grey bars) and beneath the canopy (black bars). Each bar represents a sampled population, sorted within islands in decreasing order of the proportion of seedlings recruiting beneath the canopy (see Table S1, Fig. S3 for population codes and localization). Grey boxplots show the median and the upper and the lower quartile of the proportion of seedlings outside the canopy in each island (the whiskers are 1.5 times the interquartile range from the box). Dots outside of the whiskers are considered outliers.

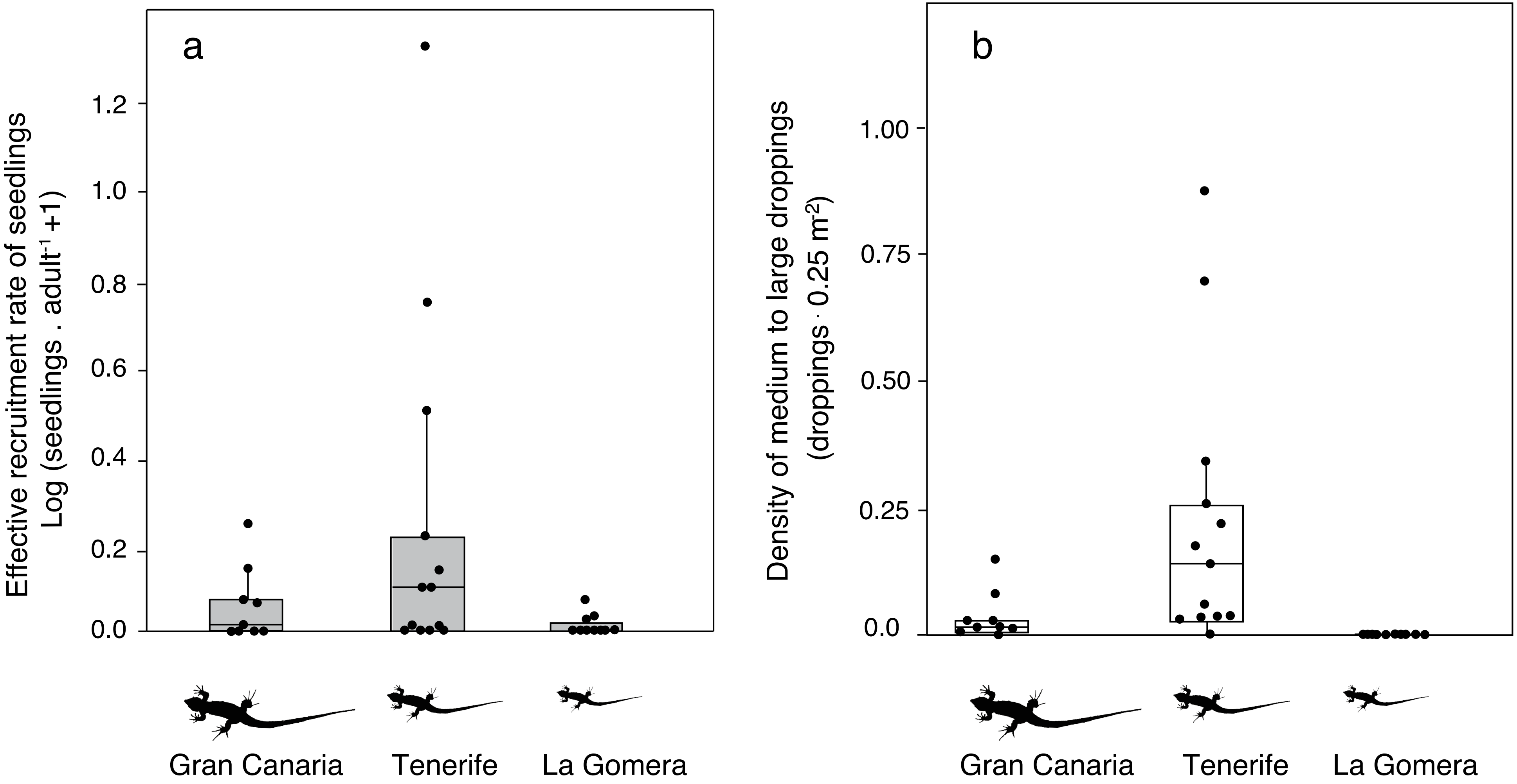
Fig. 2. Left panel (a): Effective recruitment rate of seedlings in each island (no. of seedlings outside the canopy $\cdot$ no. of adult plants^{-1}). Data are shown in *log (x+1)* scale. Right panel (b): density of large to medium droppings of lizards in each island (no. of lizard droppings $\cdot$ 0.25 m^{-2}). In both panels, population parameters are represented with points. Boxplots show the median and the upper and the lower quartile, the whiskers are 1.5 times the interquartile range from the box. Dots outside of the whiskers are considered outliers. Gran Canaria, n = 9 populations; Tenerife, n = 13 populations; La Gomera, n = 10 populations.

Fig. 3. Variation in seedling stem diameter (mm) within and among islands. Data are population mean ± S.E. (unfilled circles). At the island level, dotted lines and grey shadows indicate mean and S.E. respectively. Population codes along the abscissa, as in Table S1 and Fig. S3. Populations are ordered as in Fig. 1.

Figure


Gran Canaria
Tenerife
La Gomera
Proportion of seedlings (%)
100
80
60
40
20
1 4 6 7 2 9 8 3 5
10 12 13 15 16 21 19 18 17 14 11 20 22
24 25 26 27 29 30 32 28 23 31
Populations

Figure


a
Effective recruitment rate of seedlings
Log (seedlings . adult^{-1} +1)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Gran Canaria
Tenerife
La Gomera
b
Density of medium to large droppings
(droppings · 0.25 m^{-2})
0.0
0.25
0.50
0.75
1.00
Gran Canaria
Tenerife
La Gomera

Figure

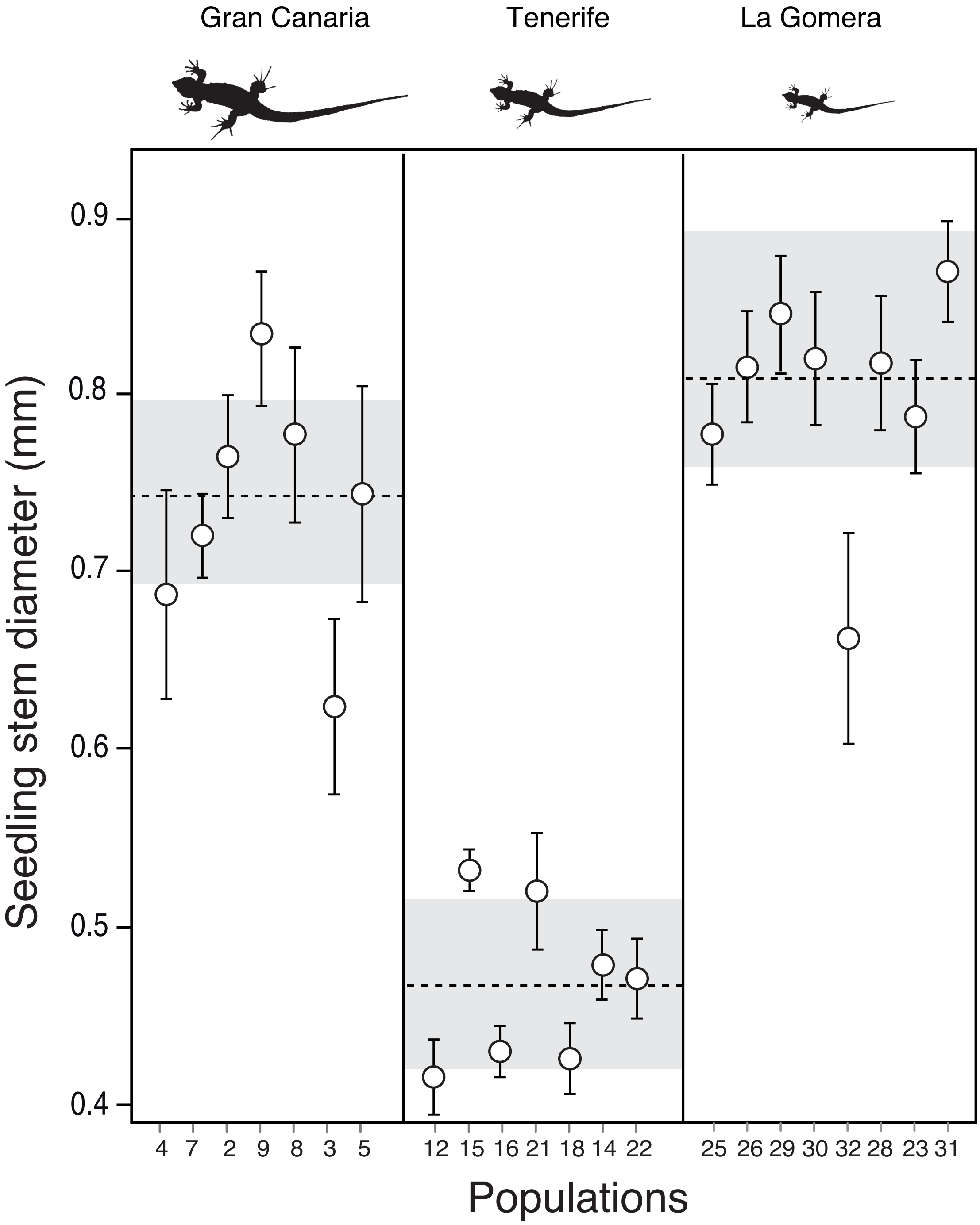

Gran Canaria
Tenerife
La Gomera
Seedling stem diameter (mm)
0.9
0.8
0.7
0.6
0.5
0.4
4
7
2
9
8
3
5
12
15
16
21
18
14
22
25
26
29
30
32
28
23
31
Populations

Table 1. Demographic parameters and results of multiple comparisons (Tukey test) among islands. The number of sampled populations is indicated within brackets. Post-hoc analyses were conducted after application of GLM[1] with binomial distribution and logit link, GLM[2] with a Poisson distribution, and log link function and LMM[3]. Non-shared, superscript letters indicate significant differences among island. Data are mean ± S.E.

Demographic parameters	**Gran Canaria**	**Tenerife**	**La Gomera**
Proportion of seedlings outside canopy[1]	12.70 ± 6.79^{a} (9)	17.71 ± 6.58^{b} (13)	2.76 ± 2.12^{c} (10)
Effective recruitment rate of seedlings[2]	0.06 ± 0.03^{a} (9)	0.39 ± 0.22^{b} (13)	0.01 ± 0.00^{c} (10)
Seedling stem diameter[3] (mm)	0.74 ± 0.06^{a} (7)	0.47 ± 0.05^{b} (7)	0.81 ± 0.05^{a} (8)