

Regeneration responses to climate and land-use change of four subtropical tree species of the southern Central Andes

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ABSTRACT

Land-use and climate change are impacting species and ecosystems around the world. The reproductive phase of plants is especially vulnerable to environmental change. Global environmental changes normally act collectively with nonlinearities and synergistic interactions being reported. Here we combined two techniques (i.e. a space-for-time substitution approach and a full factorial experiment) to assess the combined effects of climate and land-use change on the regeneration (seed production, seed germination and seedling survival and growth) of four subtropical forest tree species of the southern Central Andes (Yungas forest) in Argentina. We focus on temperature and precipitation changes and on decreased shading at the forest floor, a likely impact of changing land-use and management. We detected species-specific and phase-specific responses. Seed mass and quality was influenced by elevation (here used as a proxy for temperature change) while germination, seedling survival and growth were mainly affected by changes in the seedbed conditions (i.e. changes in shading and in moisture related to precipitation change). Despite the influence of environmental conditions on all the phases of plant regeneration studied here, we show that the major bottleneck of the regeneration phase of these species is germination. Interactive effects between the elevation of the seeds' provenance, precipitation and shading underpin the importance of studying the effects of different environmental changes collectively on plant regeneration to better forecast the effects of climate and land-use change on the Yungas forest.

1. Introduction

Terrestrial ecosystems and their associated biodiversity are strongly threatened by several human-induced changes in the environment. Land-use change was underpinned as the driver that has the largest global impact on biodiversity due to its devastating effects on habitat availability and species extinction (Sala et al., 2000). Climate change is the second most important driver of biodiversity change, mainly as a result of the expected warming of the climate system (Sala et al., 2000). These environmental changes normally do not act individually. Ecosystems are frequently under the pressure of more than one of these drivers and nonlinearities and synergistic interactions are expected (Feddema et al., 2005; Matsui et al., 2011; Perring et al., 2016; Pielke, 2005; Sala et al., 2000). However, the impacts of these interactions represent one of the biggest uncertainties in projections of future biodiversity (Feddema et al., 2005; Perring et al., 2016; Pielke, 2005; Sala et al., 2000).

Between 1990 and 2005, 66.4 million ha (or 1.7%) of the global forests were converted to other land uses (FAO and Jrc, 2012). In South

America, this conversion resulted in a loss of 2.8 and 4.3 million ha of forest per year between 1990 and 2000 and between 2000 and 2005, respectively (FAO and Jrc, 2012). Moreover, models indicate that most land-use change will continue in tropical and temperate forests of South America, mainly due to urbanization, agriculture and the increasing demand for woody biomass and biofuel (Matsui et al., 2011). Therefore, larger areas dominated by croplands and more, and larger, gaps in the forest canopy and forest edge habitats are expected in the future. Due to the significant influence of the canopy cover on the forest floor (e.g. by influencing microclimatic temperature, light and humidity), changes in land use and forest management can strongly influence the performance of species occurring in the understory.

Climate change is considered the second most important driver of biodiversity change. Global mean temperatures increased by 0.8 °C during the 20th century (IPCC, 2014), and increases of up to 5 °C are predicted to occur over the next century (IPCC, 2014). As a result of warming, precipitation amount and frequency will likely be modified (Chaoyang et al., 2012; IPCC, 2013). Moreover, the impacts of contemporary climate change have already been documented in several

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organisms and ecosystems around the world (e.g. Dillon et al., 2010; Pereira et al., 2010a; Bellard et al., 2012; Fronzek et al., 2012; Ceballos et al., 2017).

Sexual reproduction has been highlighted as one of the most sensitive phases of a plant species' life cycle. Responses of reproduction are often used as indicators of the species' sensitivity to climate and other environmental changes (Lescop-sinclair and Payette, 2016; Walck et al., 2011; Wang et al., 2006; Zhang et al., 2014). The impacts of climate change on plant species' reproduction include impacts on seed production, dormancy break, germination, and seedling establishment and performance (Gibson and Bachelard, 1986; Daws et al., 2004; Graae et al., 2009; Milbau et al., 2009; Shevtsova et al., 2009; De Frenne et al., 2011a, 2012; Mok et al., 2012; Sun et al., 2012; Carón et al., 2014). The environmental conditions, especially the temperature, experienced by the mother plant during seed production can influence seed quality by modifying morphological, chemical and physiological aspects that later determine the seeds' responses to changes in the local environmental conditions under which seeds are germinating (i.e. seedbed conditions; De Frenne et al., 2011b; Sun et al., 2012; Carón et al., 2014). Therefore, the study of seed characteristics developed under contrasting temperatures with a space-for-time substitution approach (Fukami and Wardle, 2005) can provide important information about the impacts of environmental change on seed production. Moreover, dormancy break, seed germination and seedling establishment are influenced by seedbed conditions (Hermann and Chicote, 1965; Gibson and Bachelard, 1986; Robson et al., 2009; González-rodríguez et al., 2012; Carón et al., 2014). Particularly, changes in precipitation and light (incident radiation) of the seedbed have been shown to strongly affect the success of the regeneration of tree species (González-Rodríguez et al., 2012; Quero et al., 2007; Robson et al., 2009). However, future seedbed conditions are difficult to predict and will probably be the result of a combination of the influence of climate and local forest management practices.

Here, we study the regeneration from seed of four Yungas forest tree species; *Anadenanthera colubrina*, *Enterolobium contortisiliquum*, *Jacaranda mimosifolia* and *Handroanthus impetiginosus* from populations with contrasting temperatures along an elevational gradient. The Yungas' forest is a highly biodiverse mountain forest distributed between Venezuela in the north and Argentina in the south (Brown and Kappelle, 2001a). We test for the effects of contrasting seedbed conditions (i.e. contrasting levels of precipitation and light) and specifically addressed the following aspects: (i) the variation of morphological and physiological fruit and seed traits (i.e. number of seeds per fruit, mean seed weight and seeds' viability) of trees growing under contrasting temperatures along an elevation-climatic gradient, (ii) the germination, survival and early growth of these species under different seedbed conditions (i.e. contrasting levels of light and precipitation), (iii) the presence of an interactive effect of precipitation and light and the link between the response to the changes in seedbed conditions with the temperature experienced by the mother tree. We expect that seed traits will vary with the elevation of the provenance of origin. A general increase of the seed mass with elevation is expected due to the prevalent colder conditions at higher elevations that might allow the seed to ripen more slowly with a longer filling period allowing a greater total assimilation (Fenner and Thompson, 2005). Additionally, we expect that the different phases of plant regeneration will be differently impacted by the factors analyzed here with the germination as the most sensitive phase of plant regeneration. Finally, we expect that germination, seedling survival and growth of seedlings from seeds produced at higher elevations will be more sensitive to reduced precipitation and light due to the predominant lower tree densities and higher precipitation at higher elevations. However, if heavier seeds are produced at higher elevations, the seeds and seedlings from higher elevations might have a better performance under elevated drought and shade (Fenner and Thompson, 2005).

2. Material and methods

2.1. Study area

The Yungas' forest is a highly biodiverse forest type in South America, distributed between Venezuela in the north and Argentina in the south (Brown and Kappelle, 2001a). Even though this forest type is one of the most biodiverse ecosystems of the continent (Brown and Kappelle, 2001b), it is strongly affected by forest loss, degradation and fragmentation mainly due to a strong pressure for agriculture and cattle raising during the 20th century (Brown and Pacheco, 2006; Grau and Brown, 2000; Volante et al., 2012). Nevertheless, the remaining area is extremely important for provisioning essential ecosystem services including wood production, watershed protection, and recreational benefits (Brown, 1995).

In Argentina, the Yungas cover approximately 56,000 km², are distributed between 22° and 28° latitude south, and between 400 and 3000 m a.s.l. (Malizia et al., 2012). The climate in the region is sub-tropical humid with a dry season. The mean temperature is 22 °C, while the precipitation fluctuates between 900 and 2000 mm annually varying with the elevation and the exposure of the slopes being, in general, higher in the slopes facing east. This forest contains three contrasting vegetation types associated to the elevation; Premontane Forest (*Selva Pedemontana*), Montane Humid Forest (*Selva Montana*) and Montane Forest (*Bosque Montano*; Malizia et al., 2012).

The populations considered for this study belong to the Premontane and Montane Humid Forest types and are located along an elevational-climatic gradient that extends between 23°11' and 25°4' latitude South and 64°41" and 65°32" longitude West (Table N 1), covering an elevational-climatic gradient of almost 500 m with an average difference of mean annual temperature of 3.24 °C and 286 mm of difference in annual precipitation between populations (Table 1).

2.2. Species studied

Four characteristic tree species of the Premontane and Montane Humid Forest were selected for this study: two Fabaceae species namely *Anadenanthera colubrina* (Vell.) Brenan. var. *cebil* (Griseb.) Altschul. and *Enterolobium contortisiliquum* (Vell.) Morong and two Bignoniaceae species namely *Handroanthus impetiginosus* (Mart. ex DC.) Mattos and *Jacaranda mimosifolia* D.Don. These species are distributed in the Yungas along an elevational gradient from 400 till 2000 m a.s.l (Digilio and Legname, 1966). The selected species are economically important since their wood is used for furniture and house carpentry in general (*Jacaranda mimosifolia*), for lumber to make flooring and decking (*Handroanthus impetiginosus*), for beams, frames, sleepers, posts, rods (*Anadenanthera colubrina*), for roofs, containers, shipbuilding and beehives and interior and exterior furniture (*Enterolobium contortisiliquum*). In addition, these species are frequently used in urban landscaping due to their conspicuous and colorful flowers. In terms of ecological relevance, the two Fabaceae species (*Anadenanthera* and *Enterolobium*) are important due to their capacity to fix nitrogen. Finally, flowers of all species are pollinated by insects (mostly by bees) and are frequent and conspicuous elements of the forest and the transitions to other vegetation types such as Chaco and Monte (Digilio and Legname, 1966).

2.3. Fruit and climatic data collection

Along an elevational-climatic gradient, five populations of each species were selected for fruit collection. In each population, fruits from at least ten dominant and randomly selected individuals were collected at seed maturity during their natural dispersion period between September and November of 2015.

Climatic data of the populations where the seeds were collected were extracted from Worldclim (Hijmans et al., 2005) due to the lack of

Table 1

Geographical and climatic characteristics of the populations of *Jacaranda mimosifolia*, *Handroanthus impetiginosus*, *Anadenanthera colubrina* and *Enterolobium contortisiliquum* selected for seed collection.

Species	Provenance (Codes)	Collection date	Latitude (°S)	Longitude (°W)	Elevation (m a.s.l.)	Mean annual temperature (°C)	Mean annual precipitation (mm)	Aspect ^a
<i>Jacaranda mimosifolia</i>	Orán	09/10/2015	23°11'08.41"	64°18'29.07"	349	22.29	907	North
	Salta	03/11/2015	24°46'30.1"	65°26'71"	1230	16.68	697	East
	Coronel Moldes	13/10/2015	25°05'57.23"	65°32'18.15"	1247	17.19	547	East
	La Caldera	03/10/2015	24°41'18.13"	65°23'26.95"	1251	16.34	703	Northeast
	Rosario de Lerma	17/10/2015	24°54'03.21"	65°39'42.70"	1567	15.90	503	South
<i>Handroanthus impetiginosus</i>	Coronel Moldes	13/10/2015	25°17'02.99"	65°28'49.64"	1130	17.99	482	Southeast
	Vaqueros	29/11/2015	24°42'27.27"	65°25'01.14"	1265	16.58	703	Southeast
	Cerrillos	30/11/2015	24°54'22.47"	65°28'54.76"	1272	16.69	664	North
	Chicoana	23/10/2015	25°07'30.81"	65°31'49.31"	1295	16.98	522	Northeast
	Salta	18/10/2015	24°45'44.43"	65°22'24.82"	1341	16.27	686	North
<i>Anadenanthera colubrina</i>	Güemes	01/08/2015	24°36'26"	65°4'56"	719.1	19.30	495	Northeast
	Jujuy	15/09/2015	24°19'9"	65°9'12"	1014.1	17.62	769	Southeast
	La Caldera	03/10/2015	24°41'11.95"	65°23'25.84"	1254	16.34	703	Northeast
	Chicoana	23/10/2015	25°07'22.60"	65°31'50.83"	1272	17.18	534	Northeast
	Salta	15/09/2015	24°47'03.16"	65°23'38.29"	1396	16.84	691	West
<i>Enterolobium contortisiliquum</i>	Rosario de la Frontera	15/09/2015	25°42'56"	64°59'31"	868.87	18.59	662	Southeast
	Güemes	08/09/2015	24°37'36"	65°4'11"	1027.05	19.28	490	Northeast
	Jujuy	22/09/2015	24°18'48"	65°9'36"	1027.50	17.58	776	East
	El Carril	21/09/2015	25°4'5.66"	65°29'55"	1174	17.42	576	Northeast
	Lesser	01/09/2015	24°42'50"	65°26'56"	1300.4	16.48	695	South

^a The exposure was calculated using a digital elevation model created from images captured by the Shuttle Radar Topography Mission (SRTM) with the use of QGIS.

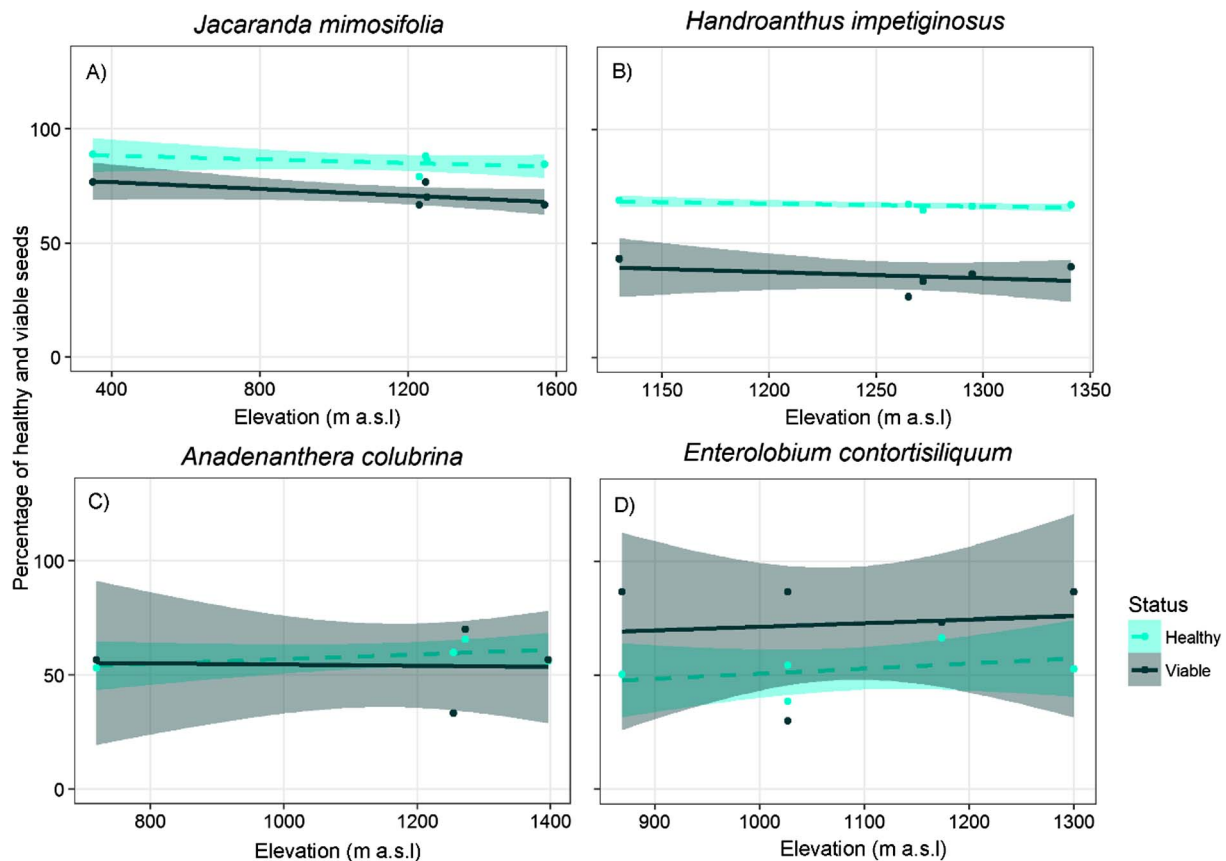


Fig. 1. Healthy and viable seeds (% of all seeds) as a function of the elevation of the provenance in (A) *Jacaranda mimosifolia*, (B) *Handroanthus impetiginosus*, (C) *Anadenanthera colubrina* and (D) *Enterolobium contortisiliquum*.

Table 2

Effects of the elevation of the provenance on the quality of fruits (number of healthy, aborted and predated seeds per fruit) and seeds (seed mass and viability). Results from GLMs.

Fruit and seed traits	Species	Parameter estimate	Standard error	z value	Pr(> z)
Healthy seeds	<i>Jacaranda mimosifolia</i>	-0.0120	0.0031	3.904	< 0.001***↓
	<i>Handroanthus impetiginosus</i>	-0.0222	0.0220	-1.010	n.s.
	<i>Anadenanthera colubrina</i>	0.0011	0.0008	1.377	n.s.
	<i>Enterolobium contortisiliquum</i>	0.0043	0.0024	1.765	n.s.
Aborted seeds	<i>Jacaranda mimosifolia</i>	0.0001	0.0001	1.170	n.s.
	<i>Handroanthus impetiginosus</i>	0.0004	0.0003	1.551	n.s.
	<i>Anadenanthera colubrina</i>	-0.0002	0.0001	-1.390	n.s.
	<i>Enterolobium contortisiliquum</i>	-0.0006	0.0002	-3.025	0.0025**↓
Predated seeds	<i>Jacaranda mimosifolia</i>	0.0019	0.0001	13.568	< 0.001***↑
	<i>Handroanthus impetiginosus</i>	0.0000	0.0002	0.083	n.s.
	<i>Anadenanthera colubrina</i>	-0.0007	0.0004	-2.126	0.034*↓
	<i>Enterolobium contortisiliquum</i>	-0.0004	0.0002	-1.595	n.s.
Seed mass	<i>Jacaranda mimosifolia</i>	-0.0007	0.0000	-22.270	< 0.001***↓
	<i>Handroanthus impetiginosus</i>	0.0024	0.0002	9.908	< 0.001***↑
	<i>Anadenanthera colubrina</i>	0.0002	0.0000	4.681	< 0.001***↑
	<i>Enterolobium contortisiliquum</i>	-0.0002	0.0001	-3.643	< 0.001***↓
Viability	<i>Jacaranda mimosifolia</i>	-0.0004	0.0005	-0.818	n.s.
	<i>Handroanthus impetiginosus</i>	-0.0012	0.0024	-0.495	n.s.
	<i>Anadenanthera colubrina</i>	-0.0001	0.0007	-0.147	n.s.
	<i>Enterolobium contortisiliquum</i>	0.0008	0.0013	0.643	n.s.

The direction of the effect is indicated by arrows: ↑ and ↓ correspond to an increase or decrease of the variables analyzed, respectively (n.s.: P > 0.05; *P < 0.05; **P < 0.01; ***P < 0.001).

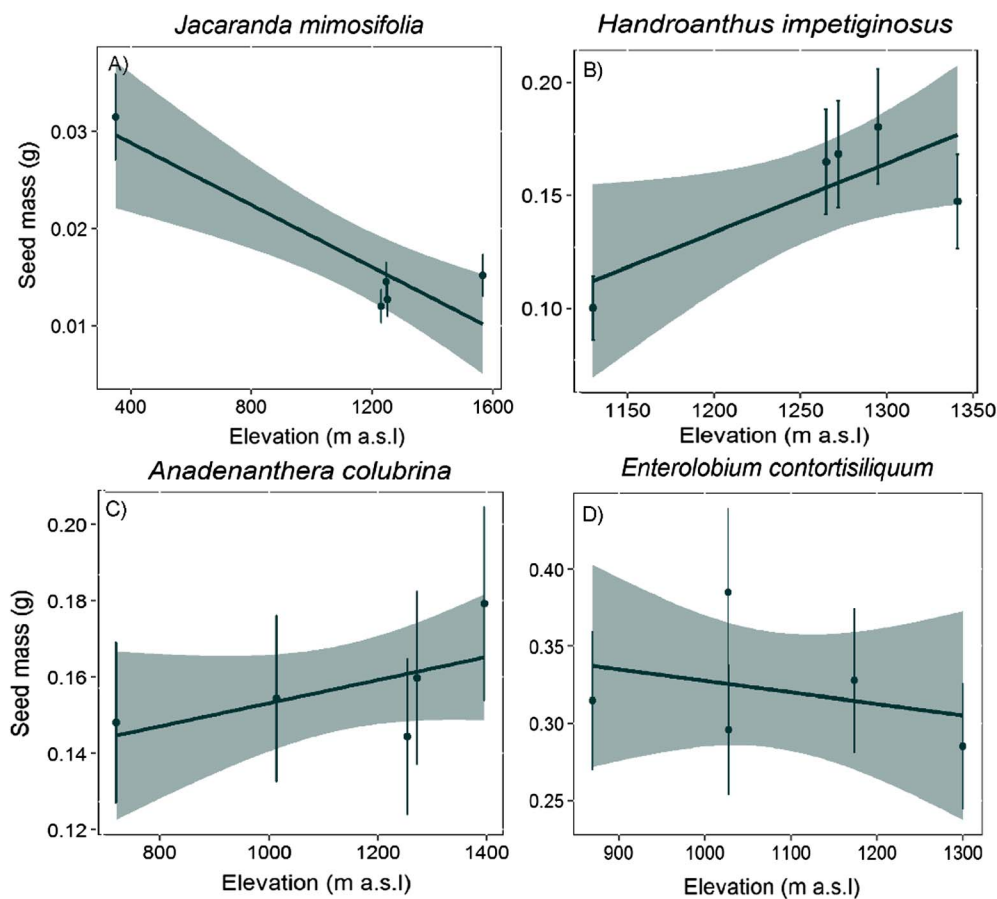


Fig. 2. Seed mass as a function of the elevation of the provenance in (A) *Jacaranda mimosifolia*, (B) *Handroanthus impetiginosus*, (C) *Anadenanthera colubrina* and (D) *Enterolobium contortisiliquum*. The error bars indicate standard errors (n = 50).

Table 3

Seed germination, mean emergence time (MET) and seedling survival and growth (total biomass and root:shoot) as a function of elevation (elev), precipitation (precip) and shading treatments (shade).

Stage	Predictor	<i>Jacaranda mimosifolia</i>		<i>Handroanthus impetiginosus</i>		<i>Anadenanthera colubrina</i>		<i>Enterolobium contortisiliquum</i>	
		LRT/scaled dev	Pr (> Chi2)	LRT/scaled dev	Pr (> Chi2)	LRT/scaled dev	Pr (> Chi2)	LRT/scaled dev	Pr (> Chi2)
Germination	Elevation	59.32	< 0.0001***↑		n.s.	28.31	< 0.0001***↑	150.13	< 0.0001***↓
	Precip (reduction)	316.16	< 0.0001***↑	104.05	< 0.0001***↑	30.31	< 0.0001***↑	40.03	< 0.0001***↑
	Shade	95.68	< 0.0001***↓	6.92	0.0315 ⁻ ↓		n.s.	40.4	< 0.0001***↑
	Elev:Precip	38.06	< 0.0001***↑	6.6	0.0369 ⁺ ↑		n.s.	16.19	0.0003***↓
	Elev:Shade	47.17	< 0.0001***↑		n.s.	8.18	0.0167 ⁺ ↑	10.35	0.0057***↓
	Precip:Shade	56.92	< 0.0001***↓		n.s.	18.71	0.0009***↓	105.51	< 0.0001***↑
	Elev:Precip:Shade	35.52	< 0.0001***↑	15.62	0.0036***↓		n.s.	29	< 0.0001***↓
MET	Elevation		n.s.		n.s.		n.s.		n.s.
	Precip (reduction)		n.s.		n.s.		n.s.		n.s.
	Shade		n.s.		n.s.		n.s.		n.s.
	Elev:Precip		n.s.		n.s.		n.s.		n.s.
	Elev:Shade		n.s.		n.s.		n.s.		n.s.
	Precip:Shade		n.s.		n.s.		n.s.		n.s.
	Elev:Precip:Shade		n.s.		n.s.		n.s.		n.s.
Survival	Elevation	14.91	0.0001***↑		n.s.		n.s.		n.s.
	Precip (reduction)		n.s.		n.s.		n.s.	13.69	0.0011***↑
	Shade		n.s.		n.s.	22.54	< 0.0001***↓		n.s.
	Elev:Precip		n.s.		n.s.		n.s.		n.s.
	Elev:Shade		n.s.		n.s.		n.s.	9.41	0.0091**↓
	Precip:Shade	32.45	< 0.0001***↓		n.s.		n.s.	15.6	0.0037***↑
	Elev:Precip:Shade	27.55	< 0.0001***↑		n.s.		n.s.	27.38	< 0.0001***↓
Total biomass	Elevation	10.56	0.0012***↑	20.28	< 0.0001***↑	8.58	0.0034**↑	6.677	0.0098**↓
	Precip (reduction)	22.85	< 0.0001***↓		n.s.		n.s.	12.045	0.0024**↓
	Shade	32.32	< 0.0001***↓	36.98	< 0.0001***↓		n.s.	47.561	< 0.0001***↓
	Elev:Precip		n.s.		n.s.	14.37	0.0008***↑		n.s.
	Elev:Shade	11.92	0.0026**↓		n.s.	8.35	0.0154 ⁻ ↓		n.s.
	Precip:Shade	12.21	0.0159 ⁺ ↑		n.s.		n.s.	9.4987	0.0498 ⁺ ↑
	Elev:Precip:Shade		n.s.		n.s.		n.s.		n.s.
Root:shoot	Elevation	7.29	0.0069**↑		n.s.		n.s.	4.5349	0.0332*↓
	Precip (reduction)	16.21	0.0003***↑		n.s.	6.01	0.0496 ⁻ ↓		n.s.
	Shade	38.56	< 0.0001***↑	14.74	0.0006***↓		n.s.		n.s.
	Elev:Precip		n.s.		n.s.		n.s.		n.s.
	Elev:Shade	16.21	0.0003***↓		n.s.		n.s.		n.s.
	Precip:Shade	23.25	0.0001***↓		n.s.		n.s.		n.s.
	Elev:Precip:Shade		n.s.		n.s.		n.s.		n.s.

The direction of the effect is indicated by arrows: ↑, ↓ and ↓ correspond to an increase or decrease of the variables analyzed, respectively. The ↓ indicates that under some combinations of the treatments there is a positive effect and under others a negative effect. (n.s.: P > 0.05; *P < 0.05; **P < 0.01; ***P < 0.001). Positive or negative effects reported for precipitation and shading means increases and decreases in the variables analyzed when reducing the precipitation and increasing shading.

more local and reliable sources of climatic information (Table 1 and Fig. 1 Supplementary material).

2.4. Seed traits

Immediately after collection, the seeds were extracted from the fruits and conserved in paper bags for 4–7 months at room temperature until the beginning of the experiment in March 2016. From a randomly selected sample of 50 apparently healthy fruits, the sanitary condition (number of healthy, predated, and aborted seeds) per species and population (five populations of each species) was recorded. The total fruit production and the sanitary condition of all the fruits produced by a mother tree was not assessed in this study. Next, the individual seed mass of apparently healthy seeds (N = 50) was determined using an analytical scale (precision of 0.1 mg).

Additionally, 30 randomly selected healthy seeds were used to determine viability. For this, the seeds were submerged in distilled water for 24 h to facilitate the extraction of the seed coat. Subsequently, the seeds were bisected through the embryo and submerged in a 0.5% Tetrazolium salt solution (The tetrazolium Subcommittee of the Association of Official Seed Analysts, 2000). After coloration (for approximately 24 h), the seeds were categorized, based on the pattern of coloration (The tetrazolium Subcommittee of the Association of Official Seed Analysts, 2000), into viable or non-viable (including seeds with

necrotic tissue).

2.5. Germination, survival and early establishment under variable levels of light and precipitation

The effects of shading and moisture availability on seed germination, survival and growth were evaluated in a full factorial common garden experiment installed at the National University of Salta (24°43'S 65°24'W and 1225 m a.s.l.) under a glass roof (without walls to allow the normal air flow and to not strongly increase the temperature compared to the open space). Three precipitation manipulation (watering) treatments based on the mean precipitation of the city of Salta for each month of duration of the experiment (March–May 2016) were applied. The treatments represented a gradient of decreasing precipitation from (i) +30% precipitation increase (mean monthly precipitation of Salta plus 30% increase); (ii) 0% no precipitation change (mean monthly precipitation of Salta city during the experiment was added); and –30% precipitation decrease (mean monthly precipitation of Salta with a reduction of 30%). The amount of water applied in each watering occasion was variable and depended on the mean monthly precipitation of each month of duration of the experiment and the treatment. The amount of water applied to the +30%, 0% and –30% was 130.6, 100.4 and 70.3 mm, 30.9, 23.7, 16.6 mm and 10.9, 8.5 and 5.9 mm in March, April, and May, respectively. The total amount of

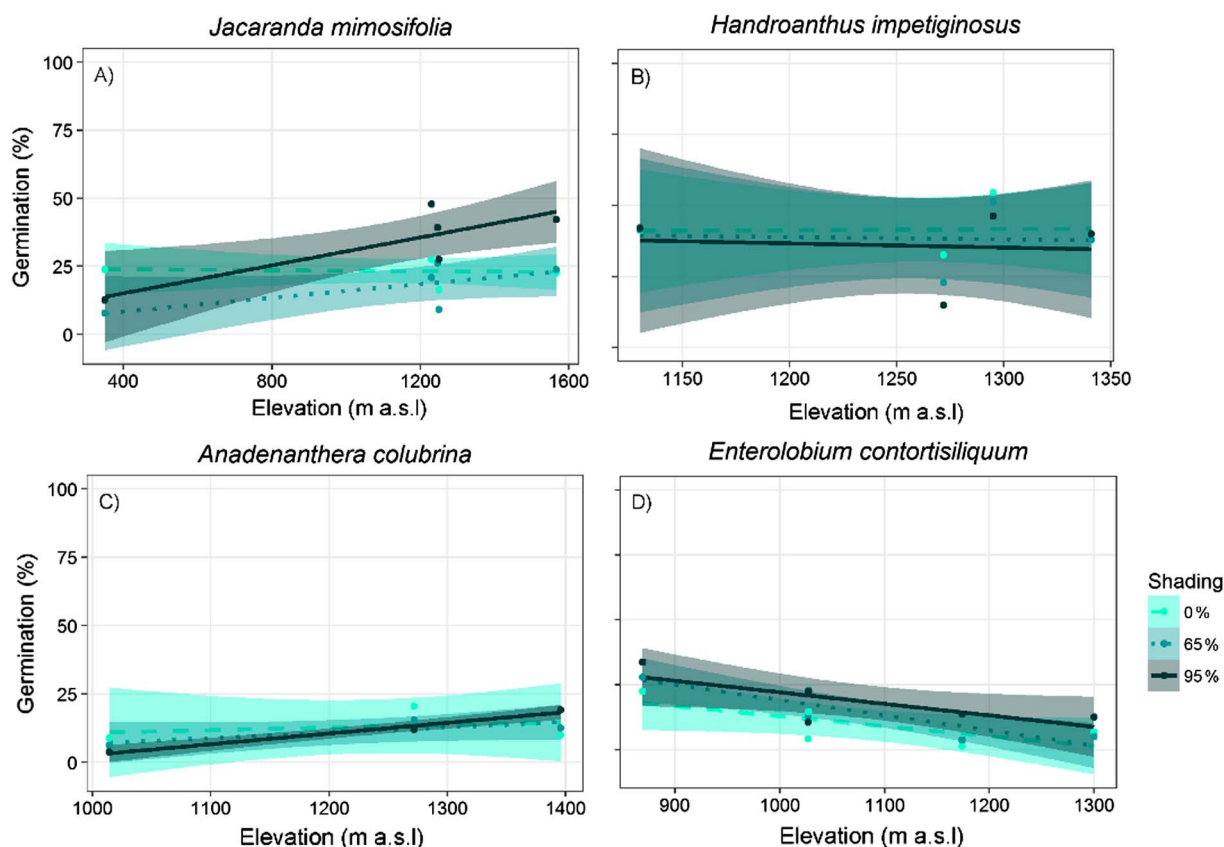


Fig. 3. Germination percentage as a function of the elevation of the provenance and the shading treatments for each of the four studied tree species (A) *Jacaranda mimosifolia*, (B) *Handroanthus impetiginosus*, (C) *Anadenanthera colubrina* and (D) *Enterolobium contortisiliquum*.

water added during the experiment was 52.9, 39.8 and 28.5 L for the +30%, 0% and –30% precipitation treatments. The shading treatments also represented a gradient of decreasing exposure to natural light and had three levels: (i) a control treatment with full exposure to natural light; (ii) 65% light reduction by using intermediately dense shade cloth and (iii) 95% light reduction by using dense shade cloth (percentage of shading produced following the manufacturer specifications).

Four replicates of 20 seeds per population (five populations of *Jacaranda* and *Enterolobium*, four populations of *Handroanthus*, and three populations of *Anadenanthera* were used due to the variable availability of seeds) were sown under each combination of shading and precipitation treatments in black polyethylene bags of 10 cm of diameter and 10 cm of height. The bags were filled with standard potting soil (humidity: 35–40%, ash 40–45%, organic matter: 15–20%, pH 7; and C/N: 7/6). Each bag was watered according to the precipitation treatment indicated above three times per week.

Because the seeds of *Enterolobium contortisiliquum* have physical dormancy, the seeds of this species were mechanically scarified by making a small cut through the seed coat before the beginning of the experiment (Janzen, 1981).

The germination (measured as the emergence of the shoot above the soil level) was recorded once per week during 70 days. After 30 days of the beginning of the experiment, the seedlings of *Handroanthus impetiginosus*, *Anadenanthera colubrina* var. *Cebil* and *Enterolobium contortisiliquum* were weeded randomly and only a maximum of four seedlings per bag were kept to avoid excessive competition between seedlings. The weeding of seedlings of *Jacaranda mimosifolia* was performed after 50 days of the beginning of the experiment because the seeds of this species germinated later.

At the end of the experiment, the germination and survival percentages under each combination of treatments were calculated for

each population and species. Additionally, the mean emergence time (MET) was calculated following formula: $MET = \sum DN / \sum N$; where D is the number of days counted from the date of sowing and N is the number of seeds that emerged on day D (Ellis and Roberts, 1980). Twenty seeds per population were used to calculate germination, while survival was calculated using the number of seedlings that remained after weeding (427, 502, 210 and 331 for *Jacaranda*, *Handroanthus*, *Anadenanthera* and *Enterolobium*). Next, the seedlings that survived at the end of the experiment (286, 463, 68 and 196 for *Jacaranda*, *Handroanthus*, *Anadenanthera* and *Enterolobium*) were carefully removed from the bags, washed to remove the soil attached to the roots and the biomass separated in the aboveground and belowground parts. Each seedling (above and belowground biomass) was placed in a paper bag and dried in an oven at 60 °C for 24 h. Finally, the above and belowground biomass were weighted with an analytical scale OHAUS Pioneer TM with a precision of 0.1 mg.

2.6. Data analysis

Due to the possible low quality of the database-extracted climatic information (cf. the heterogeneous topography of the study area and the lack of more local weather stations), the elevation of the populations was used as a proxy of the temperature experienced by the mother trees (correlation between elevation and temperature was always $r > 0.79$ and $p < 0.05$, except for the populations of *Enterolobium contortisiliquum* $p > 0.05$, Table 1 Supplementary material). Generalized linear models (GLMs) with the elevation of the provenance of the mother trees as explanatory factor were used to analyze the influence of elevation (proxy of the climatic conditions experienced by the mother tree) on the number of healthy, aborted and predated seeds (Poisson error structures) and viable seeds (binomial error structure). Generalized linear models were also used to analyze how the elevation of the

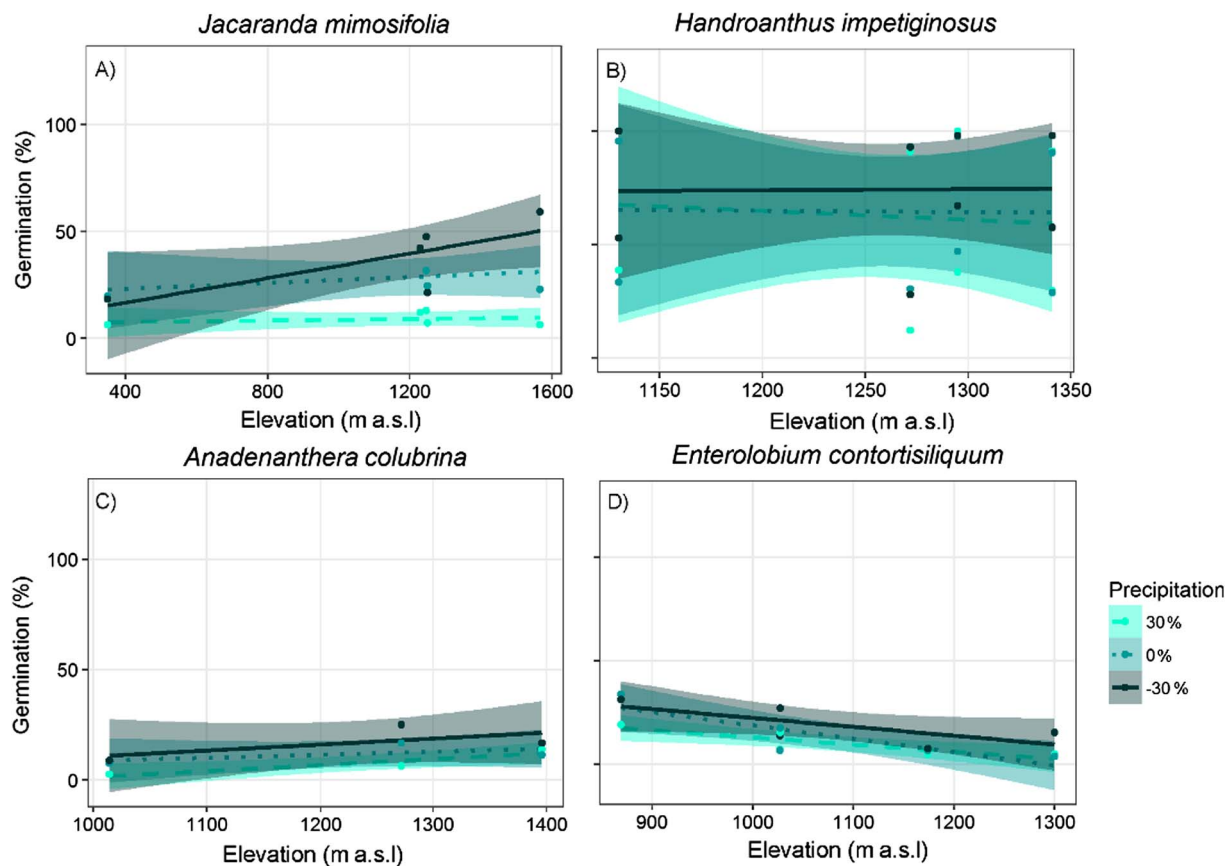


Fig. 4. Germination percentage as a function of the elevation of the provenance and the precipitation treatments for each of the four studied tree species (A) *Jacaranda mimosifolia*, (B) *Handroanthus impetiginosus*, (C) *Anadenanthera colubrina* and (D) *Enterolobium contortisiliquum*.

provenance of the mother trees, the precipitation and shading treatments (and their interactions) affect seed germination and seedling survival (binomial error structure), mean emergence time (Gaussian error structure), seedling total biomass and root:shoot ratio (Gaussian error structures). Prior to the analyses, the seed mass and the biomass variables (total biomass and root:shoot ratios) were log transformed to fulfill the normality and homoscedasticity assumptions. For each variable analyzed, first the full model was fitted (including all fixed effects and their interactions), after which models were simplified by dropping first the least significant interaction and then the least significant individual variable at each step. The comparison between models was based on the likelihood ratio test (LRT) for the binomial and Poisson data and on the scaled deviance for the Gaussian data, until all the remaining terms were significant (Zuur et al., 2009).

3. Results

3.1. Fruit and seed traits

The fruit quality (percentage of healthy, aborted and predated seeds per fruit) was influenced by the elevation but species-specific responses were observed. The percentage of healthy seeds of *Jacaranda* decreased with the elevation of the provenance while the other species did not show a significant change in the percentage of healthy seeds with the elevation of the provenance (Fig. 1 and Table 2). The percentage of predated seeds of *Jacaranda* and *Anadenanthera* increased and decreased with elevation, respectively (Table 2). On the other hand, the percentage of aborted seeds of *Enterolobium* decreased with elevation (Table 2).

The seed mass of the four species was strongly influenced by the elevation of the provenance. However, the direction of the effect varied

with the species. The seed mass of *Anadenanthera* and *Handroanthus* increased by 32% and 17%, respectively from the lowest to the highest elevation (Table 2 and Fig. 2). Conversely, the seeds of *Jacaranda* were 51% lighter at populations located at higher elevations than at lower elevations while the seeds of *Enterolobium* were 9% and 26% lighter in populations located at higher elevations than at lower elevations (Table 2 and Fig. 2).

Finally, the seed viability was not influenced by the elevation of the provenance in any of the species analyzed (Table 2 and Fig. 1).

3.2. Germination, survival and early establishment under variable levels of light and precipitation

The germination of all the species was strongly influenced by the factors and interactions considered (Table 3). However, species-specific responses were observed. The germination of *Jacaranda* and *Enterolobium* varied as a function of all factors (i.e. elevation of provenance, precipitation and light treatments) and their two and three ways interactions, while the germination of *Handroanthus* and *Anadenanthera* showed to be less sensitive to at least some of the factors and interactions (Table 3). The elevation had a major effect on germination. The germination of *Jacaranda* increased from 15% to 30% from the lower to the higher elevations, while the germination of *Enterolobium* decreased from 28% to 8% from the lower to the higher provenances. Moreover, the interactions between elevation and precipitation, and elevation and shading were significant for the germination of *Jacaranda*. Conversely, the germination of *Enterolobium* showed contrasting responses. The seeds from the lower and higher provenances germinated better under deep shade (95%) while the seeds from the provenances located at intermediate elevations exhibit the highest germination under intermediate shade (65%) (Table 3 and Fig. 3). The germination of

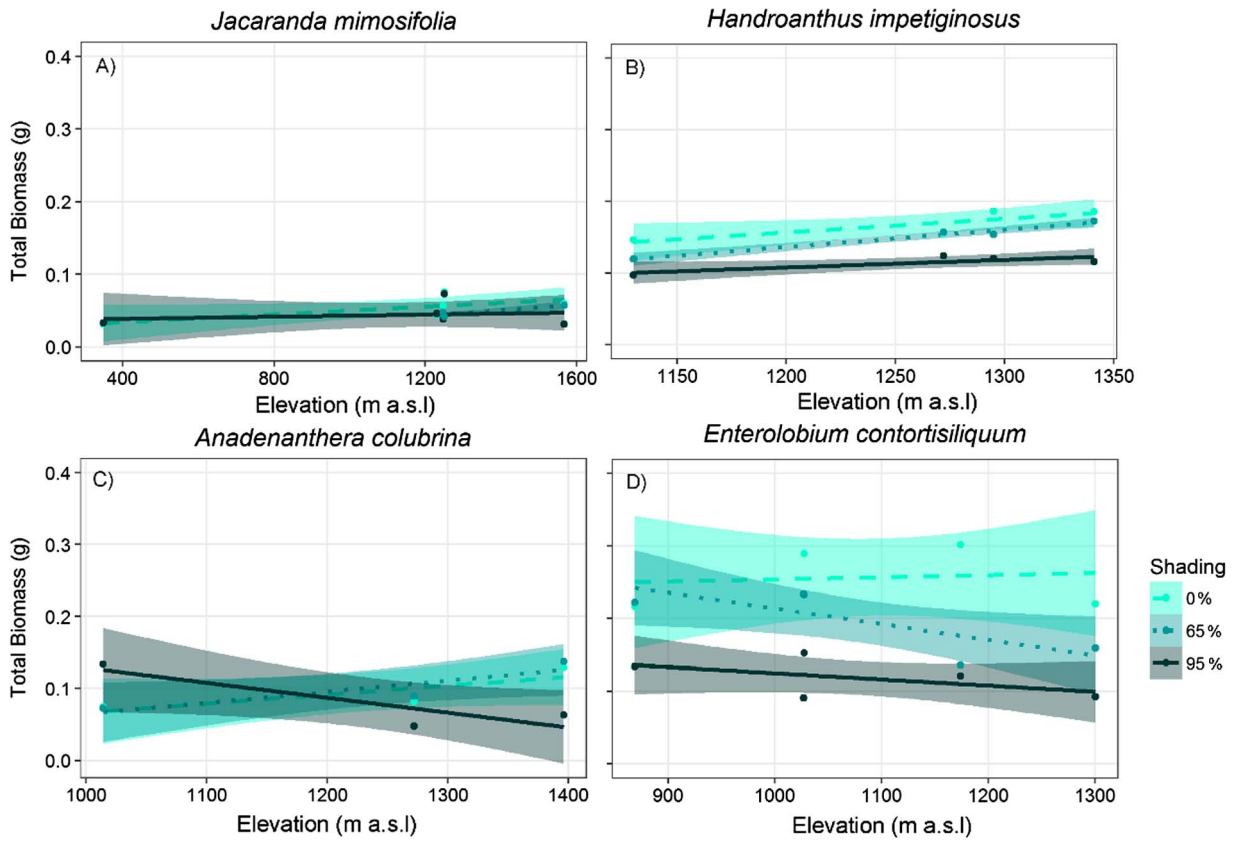


Fig. 5. Total seedling biomass as a function of the elevation of provenance of the seeds and the shading treatments in (A) *Jacaranda mimosifolia*, (B) *Handroanthus impetiginosus*, (C) *Anadenanthera colubrina* and (D) *Enterolobium contortisiliquum*.

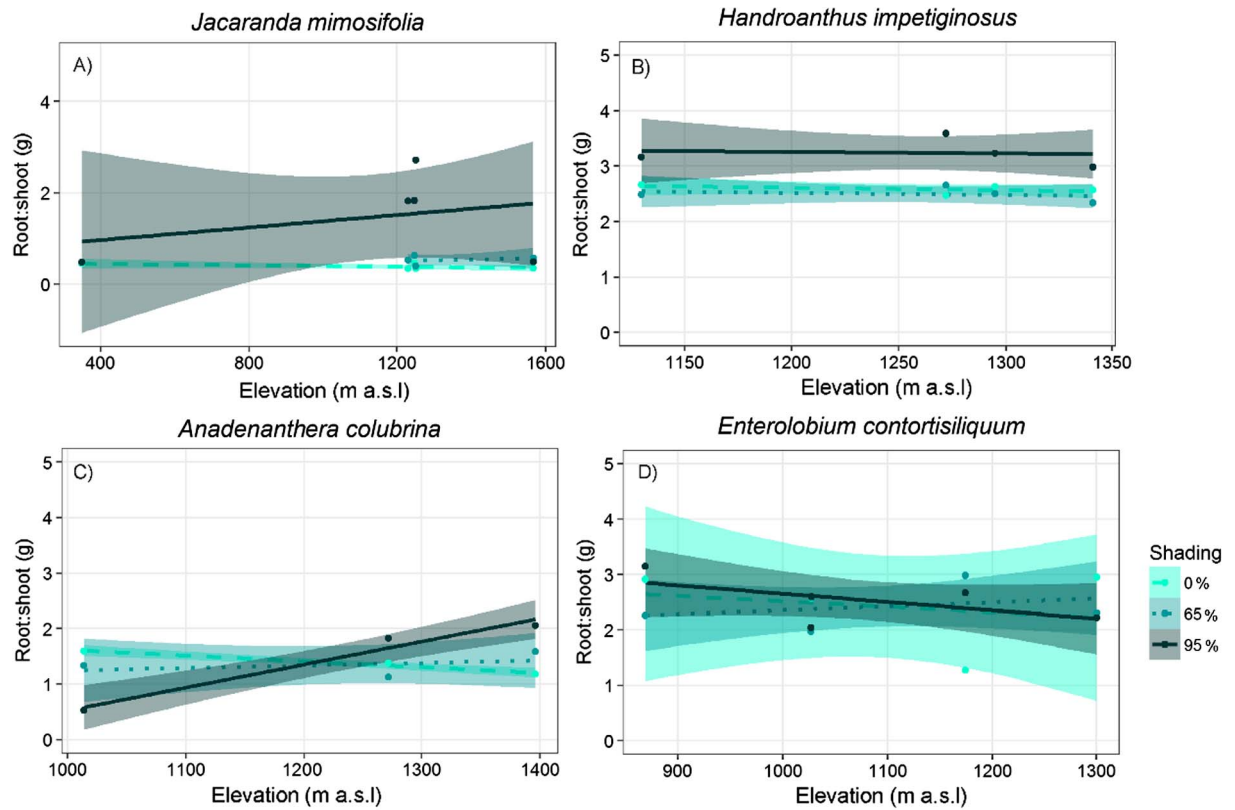


Fig. 6. Root:shoot ratio as a function of the elevation of provenance of the seeds and the shading treatment in (A) *Jacaranda mimosifolia*, (B) *Handroanthus impetiginosus*, (C) *Anadenanthera colubrina* and (D) *Enterolobium contortisiliquum*.

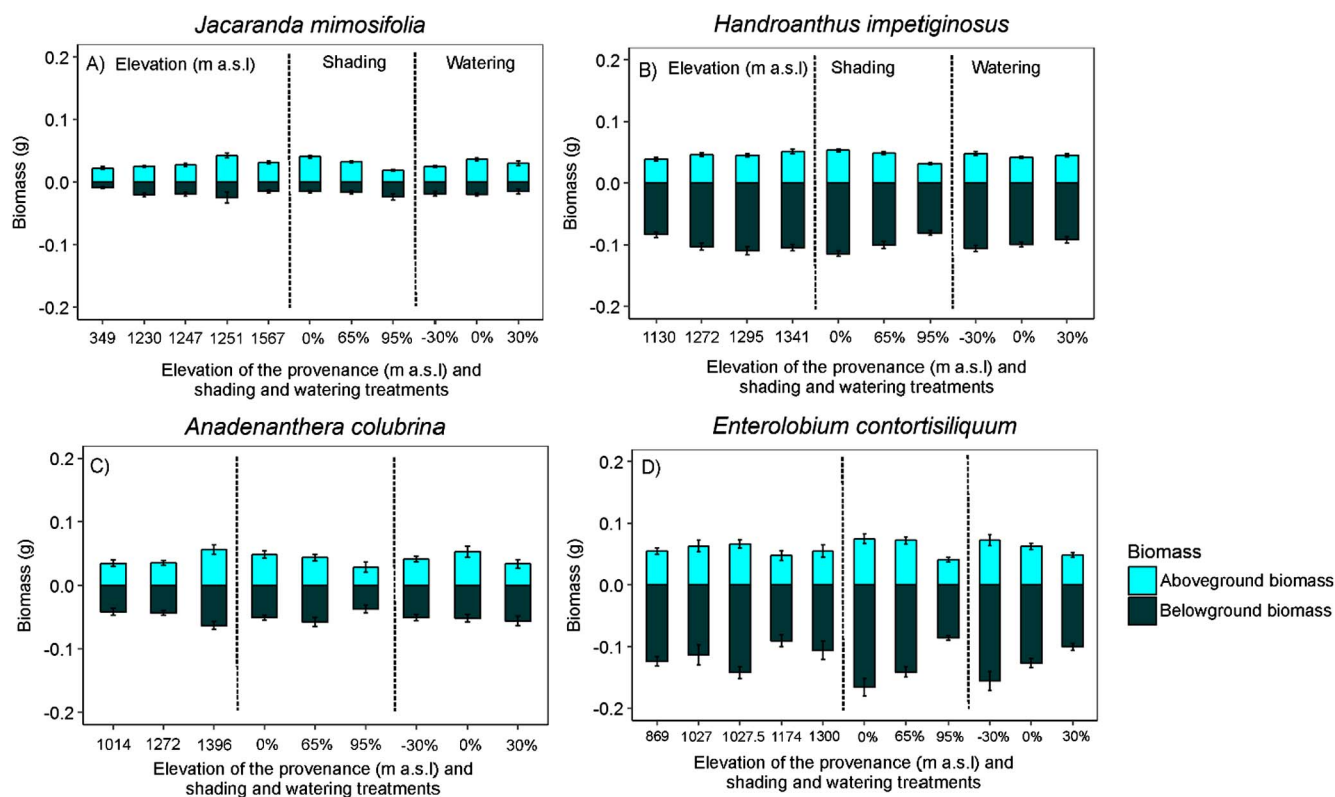


Fig. 7. Seedling below and aboveground biomass as a function of the elevation of provenance of the seeds and the precipitation and shading treatments in (A) *Jacaranda mimosifolia*, (B) *Handroanthus impetiginosus*, (C) *Anadenanthera colubrina* and (D) *Enterolobium contortisiliquum*.

Handroanthus and *Anadenanthera* was positively affected by reduced precipitation (i.e. increase of germination from 30% and 8% to 51% and 17% from the wettest to the driest conditions; Table 3). Additionally, *Handroanthus* responded positively to the interaction between elevation and precipitation (Table 3 and Fig. 4) and *Anadenanthera* to the interaction between elevation and shading (Table 3). Moreover, *Anadenanthera* showed a significant response to the interaction between precipitation and shade (Table 3); under the wettest conditions (30% higher precipitation) and driest conditions (30% lower precipitation), shading negatively affected seed germination while the opposite response was observed in seeds germinated under intermediated conditions of precipitation. The mean emergence time (MET) was not influenced by the treatments (Table 3).

Seedling survival and growth were less affected by the factors manipulated and their interactions. The survival of seedlings of *Handroanthus* and *Anadenanthera* were almost indifferent to the factors manipulated in the experiment and the elevation of the seeds' provenance with the exception of the effect of shading on *Anadenanthera* (Table 3). *Anadenanthera* exhibited a reduction of survival from 13% to 11% when increasing the shading by 95%. On the other hand, the survival of *Jacaranda* and *Enterolobium* was more sensitive to the factors and their interactions. For example, the survival decreased from 50% to 4% and from 28% to 13% from the driest and darker condition (30% reduction of precipitation and 95% shading) to the wetter condition and full sunlight exposure (30% increase of precipitation and 0% shading) for *Jacaranda* and *Enterolobium*, respectively (Table 3).

Growth was mainly affected by the individual factors, although some interactions were significant for all the species except *Handroanthus* that was only affected by the main factors (Table 3). The response of the total biomass to the elevation of provenance of the seeds and the precipitation and shading treatments was highly species-specific. *Enterolobium* showed a 10% decrease of the total biomass of seedlings produced from seeds from lower to higher provenances. Conversely, the total biomass increased by 53%, 22% and 36% from

lower to higher populations for *Jacaranda*, *Handroanthus* and *Anadenanthera*, respectively (Table 3 and Fig. 5). The levels of shading had negative effects on the seedlings total biomass of most of the species (Table 3). The seedlings were 24%, 33% and 35% smaller for *Jacaranda*, *Handroanthus* and *Enterolobium* when grown under 95% of shading compared to the seedlings grown under full sunlight exposure (0% shading) (Table 3). Finally, drought (from 30% of increase in precipitation to 0% change till 30% decrease in precipitation) decreased total biomass of *Enterolobium*. On the other hand, an increase in seedling biomass was observed for *Jacaranda* when increasing drought (from 30 increase in precipitation to 0% change; i.e. normal precipitation in Salta city) but produced the opposite effect when the increase of drought was stronger (i.e. 30% decrease in precipitation) (Table 3, Fig. 5).

The root:shoot ratio of all the species, as indicated before for the total biomass, were mainly influenced by the single factors (i.e. elevation, precipitation and shading treatments). *Jacaranda* was the most sensitive species in its biomass allocation to the changes in precipitation and shading and to the elevation of origin of the seeds as well as the two ways interactions between factors (Table 3). The root:shoot ratio of *Jacaranda* and *Enterolobium* increased and decreased respectively with the elevation of the seeds' provenance (Table 3, Figs. 6 and 7). The root:shoot ratio of *Jacaranda* and *Anadenanthera* increased and decreased 36% and 28%, respectively when precipitation was reduced by 30% (Fig. 7). Finally, the biomass allocation of *Jacaranda* and *Handroanthus* was influenced by the shading treatments (Table 3). *Jacaranda* showed an increase of ratio when light was decreased, indicating more belowground allocation while *Handroanthus* showed divergent responses (i.e. increase and decrease of root:shoot ratio depending on the level of shading applied; Table 3, Figs. 6 and 7).

4. Discussion

In this study, we not only modified seedbed conditions by shading

and precipitation manipulations but also assessed the influence of the climatic conditions experienced by the mother tree (elevation as proxy for temperature) to assess the possible impacts of climate and land-use changes on the regeneration and early establishment of four important forest tree species of the Yungas forest in South America. We found that the environmental conditions experienced by the mother tree and the seedbed conditions strongly influence most of the regeneration phases (except seed viability and MET). However, species-specific and phase-specific responses were frequent (Milbau et al., 2009; Shevtsova et al., 2009; Carón et al., 2014).

The influence of the environmental conditions experienced by the mother tree was highly variable between seed traits and species. The seed trait most strongly affected by the environmental conditions experienced by the mother trees along the elevational gradient was seed mass. The seed mass is a key aspect for plant regeneration because it represents the amount of resources available for the first stages of seedling establishment (Fenner and Thompson, 2005). However, not all the species showed the same response; *Handroanthus* and *Anadenanthera* showed an increase of seed mass with elevation probably linked to the decreasing temperatures with increasing elevation (negative correlation between temperature and elevation). The production of heavier seeds at lower temperatures is linked to slower seed ripening, which allows the seed filling process to occur more slowly allowing for a greater total assimilation (Fenner and Thompson, 2005). On the other hand, *Jacaranda* and *Enterolobium* showed the opposite behaviour (i.e. heavier seed produced at lower elevations). For these species, probably other factors not considered here, such as genetic variability (Castro et al., 2008; Tíscar Oliver and Lucas Borja, 2010), have a stronger influence on the seed mass than the temperature. Nevertheless, these species-specific responses are not uncommon. For instance Carón et al. (2014) showed that closely related *Acer* species exhibit divergent responses of seed mass to temperature variation. Surprisingly, the seed viability was not influenced by the elevation therefore, and based on the general significant correlation between temperature and elevation, it is likely that this aspect will not be affected by changes in temperature in the future. However, if the temperatures in the future exceed the maximum temperature considered here this aspect might be impacted as was reported in other species (Carón et al., 2014; Verheyen et al., 2009).

After seed production, seed germination was the most sensitive phase of the regeneration to changes in environmental conditions independently of the species considered. However, species-specific responses were frequent as reported before for other species (Castro-Marín et al., 2011; Carón et al., 2014). The species more sensitive to environmental changes (i.e. precipitation, shading and the elevation of the provenance of the seeds and their interactions) were *Jacaranda* and *Enterolobium*. The elevation of the provenance of origin of the seeds showed to have a positive effect on the germination of *Jacaranda* and *Anadenanthera* as indicated before for other species (Caliskan, 2014; Carón et al., 2014). Additionally, the germination of all species was negatively influenced by increasing precipitation as reported before for other tree species (Carón et al., 2014). This negative effect of increasing precipitation on germination might be related to the fact that moist conditions might facilitate the development of pathogens (Blaney and Kotanen, 2001) negatively affecting seed germination. However, for other species such as *Fagus sylvatica* and *Quercus ilex* the opposite response was reported (Pérez-Ramos et al., 2013; Thiel et al., 2014). These contrasting responses are probably related to the species' drought tolerance and the environmental characteristics of the forest where the species are naturally present. For the species under study, the observed response (negative effect of increasing precipitation on seed germination) indicates that germination will be negatively impacted by enhanced precipitation. Additionally, the two and three ways interactions between precipitation, light and elevation of provenance of the seeds had an important influence on seed germination, especially for *Jacaranda* and *Enterolobium*. For example, for *Jacaranda* the germination was

significantly higher for seeds from higher populations under driest conditions compared with the seeds from lower populations germinated under wetter conditions. This is likely related to the negative correlation between precipitation and elevation reported for this species and the possible adaptation of the mother plants producing seeds under drier conditions.

Seedling survival and seedling growth were less strongly influenced by the elevation of the provenance of the seeds (Carón et al., 2015) and changes in the seedbed conditions (changes in precipitation and shading) than the germination. Elevation basically lost all influence on seedling survival and growth indicating that the provenance was mainly important for seed quality and seed germination. This contradicts earlier findings where a strong influence of provenance to changes in seedbed conditions was reported not only for seed germination but also seedling survival and growth (McCarragher et al., 2011).

The survival of *Handroanthus* seedlings was indifferent to all the factors and interactions, this species survival was not affected by changes in the seedbed conditions or the elevation of the seeds provenance. Therefore, this species will likely be less impacted by changes in environmental conditions than the other species considered here providing *Handroanthus* with competitive advantages under future conditions. On the other hand, *Jacaranda*, *Anadenanthera*, and *Enterolobium* were mainly influenced by the interactions between factors (i.e. the response to changes in one variable interact with the changes on other variables) evidencing the complexity of the survival phase.

Seedling biomass was mainly affected by individual factors. Seedlings total biomass decreased with increasing shading (from 0% reduction to 95% reduction of direct sunlight) as reported before for other species (Löf et al., 2005), a decrease that is likely caused by the reduced photosynthesis in darker (more shaded) environments (Osunkoya et al., 1994). The precipitation treatment showed to be relevant only for *Jacaranda* and *Enterolobium* but with divergent responses. *Jacaranda* showed increases and decreases of biomass when increasing precipitation while *Enterolobium* increased its biomass when increasing precipitation. Therefore, under wetter conditions *Enterolobium* will, most likely, exhibit competitive advantages in terms of biomass development.

Finally, *Jacaranda* invested more in belowground biomass than in aboveground biomass under dry conditions, as found in other species (Kramer et al., 2010; Dreesen et al., 2012; Carón et al., 2014). This allocation of resources in favour of belowground biomass is likely related to the need of develop more roots to reach a bigger volume of soil and therefore more water. Conversely, *Anadenanthera* showed the opposite behaviour probably related to the drought tolerance of this species. Moreover, species-specific responses to light were observed regarding biomass allocation. For example, *Jacaranda* increased the root:shoot under darker conditions evidencing there was less aboveground biomass production in the shade relative to belowground biomass (see Löf et al., 2005).

5. Conclusions

Our findings indicate that *Jacaranda* and *Handroanthus* regeneration may positively react to enhanced drought and negatively to increasing forest gaps, while *Anadenanthera* and *Enterolobium* regeneration may be positively impacted by drought and increasing light. Considering that the species studied here naturally regenerate in the wet season (summer in the northwest of Argentina) and that climate change projections indicate that the contrast between dry and wet seasons will increase in the future, it is likely that, if the precipitation increases in the wet season, these species will be negatively impacted in the future. However, these findings should be considered with caution because under natural forest conditions there are also other factors such as canopy interception and evapotranspiration that might influence the seedbed conditions and soil moisture content. More research on these matters is needed to better predict the future regeneration of these

species in the face of climate change.

According to our findings, and considering the increase of germination, survival and seedling biomass with elevation and the positive interaction between precipitation and elevation recorded for *Jacaranda*, *Handroanthus* and *Anadenanthera* we suggest that the best locations for seed collection for forestry purposes are the populations located at higher elevations. Considering that a general opposite response was recorded for seeds of *Enterolobium*, we suggest that seeds of this species should be collected in populations located at lower elevations to increase the chances of reproductive success of these species under future environmental conditions such as the simulated here. The species-specific and phase-specific responses observed here concerning seed morphological and physiological aspects, seed germination and seedling performance point out the complexity of interactions between provenance and seedbed conditions on plant reproduction (Benech Arnold et al., 1995; Marrush et al., 1998; Zerche and Ewald, 2005). Moreover, it was surprising to find that the temperature experienced by the mother tree during seed production had divergent impacts on the different seed traits analyzed and strongly varied between species. Even though all the phases of reproduction were influenced by several abiotic factors, the seed germination under a combination of various provenance and seedbed conditions was the main bottleneck phase for these species. Most likely, this is the phase of plant regeneration that will be most strongly affected by climate and land-use change and forest management. Moreover, here we point out the importance of not considering reproduction as a single process and we suggest that is necessary to study individually each stage of plant regeneration when analysing future scenarios of populations' dynamics. Considering the frequent divergent and species-specific responses of seeds and seedlings to the temperature experienced by the mother tree and the seedbed conditions we advise to investigate these responses further, also in other species. Due to the strong pressure of deforestation in the study area, this work provides key information for forest management and restoration plans to better select adapted provenances and locations to assure a successful regeneration of these species.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foreco.2018.02.006>.

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