

Phylogenetic relatedness and competition: a pot experiment with semiarid tree species

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ECOLOGY

ABSTRACT

Background: Factors determining coexistence of the species have been recognized out since Darwin, but empirical studies have brought the relationship between niche similarity and competition into question. Knowing the mechanisms of coexistence makes it possible to predict biological invasions and determine better species combinations for the restoration and recovery of degraded areas. This study aimed to test the competition-relatedness hypothesis for semiarid tree species. Thus, an experiment was carried out with a phylogenetic gradient of tree species, planted in pairs. The phylogenetically closest pair consisted of congeneric species of the same clade, the intermediate pair comprised congeneric species of distinct clades, and the more distant pair were species of the same family.

Results: Our results show that the phylogenetically closest and the more distant pairs corroborated the competition-relatedness hypothesis, but the presence of one species with high competitive ability in the intermediate pair caused an unexpected pattern.

Conclusion: Niche differences are important for coexistence, but it is necessary to know the competitive ability of each species and the specific associations that are more productive to improve the efficiency of restoration programs and to reveal the degree of aggressiveness in the case of invasive species.

Keywords: functional traits, facilitation, *Cordia oncocalyx*, *Cordia glazioviana*, *Mimosa tenuiflora*, *Mimosa caesalpinifolia*, *Libidibia férrea*, *Poincianella bracteosa*

HIGHLIGHTS

Close phylogenetically tree species from the semiarid region have functional similarities.
Close phylogenetically related tree species compete more than distant species.
Phylogenetically distant species show facilitative interaction.
Competitive ability must be considered to determine the effects of species interactions.

TAVARES, M. T., DE SOUZA, B. C., MENEZES, B. S., ARAÚJO, F. S., SFAIR, J., ZANDAVALLI, R. B. Phylogenetic relatedness and competition: a pot experiment with semiarid tree species. CERNE, v.28, e-102819, doi: 10.1590/01047760202228013036

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Received: 03/12/2021

Accepted: 10/05/2022



INTRODUCTION

Over time, ecologists have attributed the maintenance of biological diversity to mechanisms that limit niche overlap (MacArthur; Levins, 1967; Webb *et al.*, 2002; Cavender-Bares *et al.*, 2009; Kraft *et al.*, 2015). This diversity enables coexistence and reduces the probability of competitive exclusion, ensuring that each species exhibits preferences for specific resources, in such a way that they will be more limited by intraspecific competition than by competition with individuals of other species (Chesson, 2000).

Based on theories that involve niche similarity and increased competition, one recurrent hypothesis is that closely related species are less likely to coexist due to a considerable functional similarity (Webb *et al.*, 2002; Cahill *et al.*, 2007; Cavender-Bares *et al.*, 2009). This hypothesis, known as the competition-relatedness hypothesis (CRH), assumes that there is conservatism of functional traits along a lineage, making functionally related species more similar than phylogenetically distant species (Webb *et al.*, 2002). Here, functional similarity means similar traits in resource acquisition and use, which leads to niche overlap and competition.

Studies have already been carried out to test the CRH, and divergent results have been found. While some studies corroborate the CRH (Straus *et al.*, 2006; Maherali, Klironomos 2007; Violle *et al.*, 2011), others have found that competition was more intense between distant taxa than between closely related taxa (Cahill *et al.*, 2007; Narwani *et al.*, 2013; Alexandrou *et al.*, 2015; Lyu *et al.*, 2017). The results depended on the species group selected: in species with similar competitive ability, coexistence depended only on the niche differences, so that closely related species and those with more similar niches competed more. On the other hand, when the species group selected included one or more species with high competitive ability, coexistence also depended on competitive ability, and the highly competitive species suppressed the others, closely related or not (Mayfield, Levine 2010; Cahill *et al.*, 2007). Superiority in competitive ability is evident in different traits related to rapidity and the efficiency of light and soil resource acquisition, i.e., traits related to high growth rates (Godoy 2019).

However, evolutionary relatedness may not be the only explanation for species to share functional traits. They are also influenced by abiotic factors, which select for traits that are better adapted to the physiological requirements of the environment (Lossos 2008; Kraft *et al.*, 2015). Thus, phylogenetically distant species that have evolved in the same environment can have similar traits that permit them to persist under those conditions and resource levels (called functional convergence), and closely related species that have evolved in different environments can be functionally divergent (Webb *et al.*, 2002; Kraft *et al.*, 2015).

Both competition and abiotic factors select species that will coexist, and drive community structure, generating phylogenetic patterns in the species composition. A community structure with a phylogenetically closer species pattern (phylogenetic aggregation) is found when traits are conserved among related species and abiotic conditions are the filtering process by which functionally similar taxa are selected, being more able to survive under the conditions imposed (Hoiss *et al.* 2012). In contrast to the phylogenetic aggregation pattern caused by environmental filtering, competition can lead to a phylogenetically disperse pattern (phylogenetically distant species) in community structure. If traits are conserved among related species and competition is filtering the species composition, functionally and phylogenetically distant species will remain in the community (HilleRisLambers *et al.* 2012). Another factor that drives phylogenetic dispersion is the positive interaction. The most common kind of positive interaction among plants is facilitation, where an adult plant, called a nurse plant, increases germination and growth of other species by environmental amelioration. It has been shown that facilitation can occur between highly phylogenetically distant species, creating a pattern of phylogenetic dispersion in the community structure (Verdú *et al.* 2012).

To test the CRH hypothesis, it is necessary to induce species interaction among species with different degrees of similarity. Competition is investigated by different methods, mainly field and pot experiments. Manipulation is a feature of both approaches, and variation in the density of individuals is a common technique. Frequently, species growing alone (intraspecific treatment) are compared to two (or more) species growing together (interspecific treatment). It has been demonstrated that individuals grow better alone if the other species in the interspecific treatment is a strong competitor, or grow worse alone if the other species in the interspecific treatment has facilitative effects (Burn, Straus 2012). In experiments which compare intra- and interspecific interactions it is expected that competition will be more intense in the intraspecific interaction, due to greater niche similarity (Chesson, 2000), but the result strongly depends on the competitive ability of the species involved in the interspecific interaction (Cahill *et al.*, 2007).

Within this theoretical framework we are interested in understanding the consequences of ecological interactions among species in a phylogenetic gradient, to test the CRH. Our specific aims are to determine: (i) if phylogenetically closely related species are also functionally similar, primarily in traits related to water use; (ii) if there is phylogenetic conservatism among the species; and (iii) if the interaction between phylogenetically closely related species is competition and between distantly related species is facilitation.

MATERIAL AND METHODS

Study conditions and species selection

The experiment was conducted in a greenhouse at the Federal University of Ceara (located in the municipality of Fortaleza, Ceara, Brazil; 3°48'26.60"S 39°21'8.40"W; 1448 mm average rainfall; 26.3°C average temperature). The species selected for the study are commonly found in vegetation locally known as *Caatinga*, which can be classified as Seasonally Dry Tropical Forest (SDTF; Pennington *et al.*, 2009; Oliveira *et al.*, 2013). *Caatinga* vegetation is predominant in the Northeast region of Brazil, where the climate is semiarid and the main characteristic is seasonality in water availability due to drought events that vary annually in intensity and duration (Sampaio, 1995). Seedlings and seeds of the species were collected in SDTF remnants located at the Vale do Curu Experimental Farm in the municipality of Pentecoste, Ceara, Brazil (3°48'S, 39°21'W; 759 mm; 28.3°C) and in the private nature reserve *Não Me Deixes* Farm, in the municipality of Quixadá, Ceara, Brazil (4°49'S, 38°58' W; 765 mm; 27.1°C).

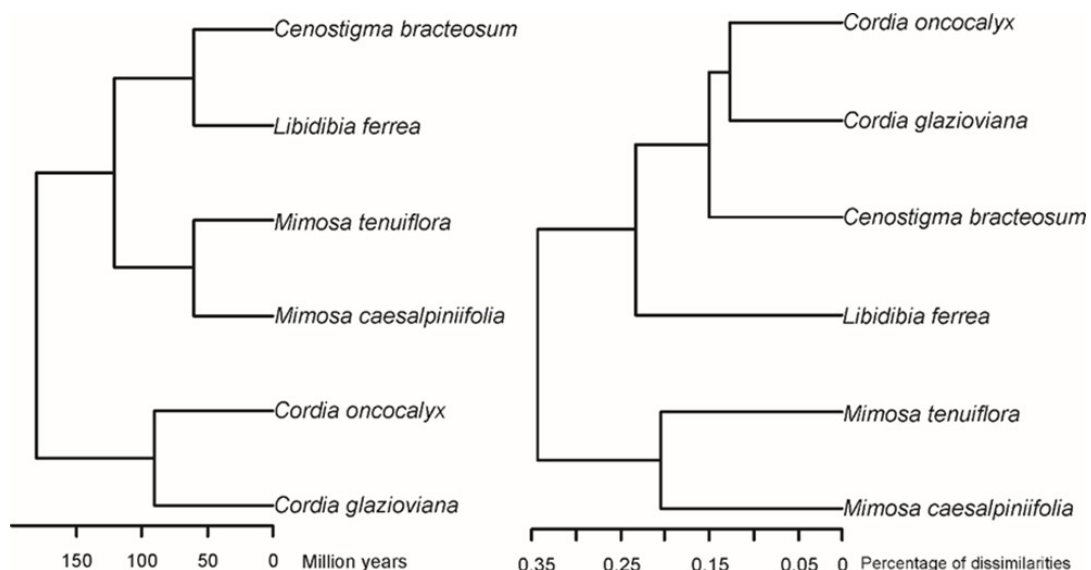
To test the CRH we used a phylogenetic relatedness species gradient for the interaction experiment. The gradient was: close species pair (close), intermediate pair (intermediate) and distant pair (distant). The close pair consisted of two species of the same genus that belong to the same clade, signifying that they have recently evolved from a common ancestor. The intermediate pair was composed of two species from different clades of the same genus, signifying that their last common ancestor was less recent. Finally, the distant pair included species from different genera but from the same family. The species selected were: close pair, *Cordia oncocalyx* Allemão and *Cordia glazioviana* (Taub.) Gottschling & J.S. Mill; intermediate pair, *Mimosa tenuiflora* (Wild) Poir. and *Mimosa caesalpinifolia* Benth; and distant pair, *Libidibia ferrea* (Mart. ex Tul.) L. P. Queiroz

and *Cenostigma bracteosum* (Tul.) E. Gagnon & G.P. Lewis (Figure 1). *C. oncocalyx* and *C. glazioviana* belong to the Boraginaceae family, *M. tenuiflora* and *M. caesalpinifolia* belong to the Fabaceae family and Mimosoideae subfamily, *L. ferrea* and *C. bracteosum* belong to the Fabaceae family and *Caesalpinioideae* subfamily. We selected these species because they fulfilled the following requirements: they comprised a phylogenetic gradient, co-occur in caatinga vegetation, and seeds or seedlings were available.

Experimental design

After germination the young plants were maintained in germination trays for 20 days before being transplanted into 8 L pots containing a substrate composed of native soil and earthworm humus at a ratio of 2:1 (v:v). After this period, plants of similar height were randomly placed in the following treatments: alone (one individual), intraspecific interaction (two individuals of the same species) and interspecific interaction (one individual of both species of either the close, intermediate or distantly related pairs), each with 20 replications. The interspecific treatments were: *C. oncocalyx* with *C. glazioviana*, *M. tenuiflora* with *M. caesalpinifolia* and *L. ferrea* with *C. bracteosum*. The plants were maintained for 120 days and watered daily to prevent water stress. The plants were positioned equidistant (8.33 cm) to one another and to the edge of the pot. When alone, the seedling was placed in the center of the pot. In each treatment, the 20 replications were separated according to measurement type: (a) continuous non-destructive; five pots to measure maximum photosynthesis rate (A_{max}), stomatal conductance (g_s) and transpiration rate (E) and five pots to measure leaf longevity; (b) continuous destructive; five pots to measure leaf water potential at predawn (Ψ_{PD}) and at midday (Ψ_{MD}); and (c) non-continuous destructive; five pots to determine leaf morphological traits and total biomass.

Figure 1. Species phylogenetic (a) and functional (b) dendrogram. Phylogenetic tree was based on Simon *et al.* (2011).



Leaf functional traits

To determine the physiological traits we measured the maximum rate of photosynthesis (A_{max} ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s ; $\text{mol m}^{-2} \text{ s}^{-1}$) and intrinsic water use efficiency (A_{max}/g_s ; $\mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$). These traits were measured at 48-hour intervals for 10 days, using a portable infrared gas analyzer (Licor LI-6400XT). One leaf from each individual (five individuals per treatment) was kept in the meter chamber for approximately 120 s with a fixed CO_2 concentration of 400 ppm and a flow rate of $400 \mu\text{mol s}^{-1}$. Radiation inside the chamber was fixed at $1500 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ using an artificial light source (LI-6400XT LED). A_{max} , g_s and WUE measurements were taken when the species had highest photosynthetic activity, which was ascertained before the experiments began. The light response curves were constructed using the automatic light response program of the LI-COR 6400XT system (see Evans & Santiago 2014). Three leaves for each species were maintained in the IRGA chamber for 200 minutes under different light intensities (0, 20, 50, 100, 200, 500, 1000, 1500 and $2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$) (Supplementary data S1).

We also measured leaf water potential at predawn (Ψ_{PD} ; MPa; between 00:00 and 4:00 a.m.) and midday (Ψ_{MD} ; MPa; between 12:00 and 13:00 p.m.) using a Scholander pressure chamber (model P3005F01, Soil Moisture Equipment, California, USA). One leaf per individual was measured, totaling five measurements per treatment on each measurement day. The measurements were taken on five occasions; once every 48h for 10 days. To understand the hydraulic strategies, it was necessary to simulate drought conditions and then evaluate the water potential behavior. So, irrigation was ceased in the treatments, and the leaf water potential was measured under drought conditions until total leaf loss occurred, a duration of four to six months, depending on the species. Ψ_{PD} and Ψ_{MD} were used to evaluate the hydraulic strategies of each species in response to the loss of conductivity under drought conditions (Martinez-Vilalta *et al.*, 2014). This approach consists of creating a linear regression between Ψ_{PD} and Ψ_{MD} . In the regression, the slope value (σ) categorizes the species based on the degree of stomatal sensitivity to drought, while the intercept value (Λ) represents the maximum rate of transpiration per unit of transported water. The σ value is analogous to the stomatal opening behavior of plants. If $\sigma = 0$ the species is isohydric, adjusting their stomatal opening to maintain midday leaf potential relatively stable as soon the soil dries (constant leaf potential as soil potential declines), if $\sigma = 1$ the species is anisohydric and has a less strict stomatal control (the difference between leaf potential and soil potential stays constant). Λ signifies the transpiration flow relative to the plant hydraulic capacity under well-watered conditions (equivalent to the leaf water potential at $\Psi_s \approx 0$) (Martinez-Vilalta *et al.*, 2014).

The turgor loss point (πTLP) was also measured as an indicator of water stress and drought tolerance (Bartlett

et al., 2012). To determine πTLP , pressure-volume curves were constructed using the bench-drying method. Three leaves from three individuals of each species were used to create the curves. The leaves were hydrated in water overnight, then weighed, and their water potential was measured. They were then placed on the bench to dry and were weighed, and water potential was measured, every two hours until the water potential became constant. The dry leaf weight was measured and the relative water content was calculated. Pressure-volume curves were constructed for each species from the sequential relative water content and water potential measurements (Tyree; Hammel, 1972; Bartlett *et al.*, 2012). Species with higher πTLP s avoid excessive water-loss, do not expose their tissues to large negative pressure and have better chances of continuing to function under drought conditions (Bartlett *et al.*, 2012; Martinez-Vilalta *et al.* 2014).

The following leaf traits were also measured: specific leaf area (SLA; leaf area / dry mass, $\text{mm}^2 \text{ mg}^{-1}$), relative water content (RWC; fully saturated leaf mass – dry leaf mass / dry leaf mass, g g^{-1}), leaf dry mass (LDM, mg) and leaf longevity under drought (LL, days). To make these measurements, five fully expanded mature leaves with no visible injury were collected from each individual. The leaves were stored in a refrigerated thermal box after collection, to avoid water loss, as detailed by Pérez-Harguindeguy *et al.*, (2013) in their protocol for the measurement of leaf functional traits. In the laboratory, leaf fresh mass was measured with a precision scale (Mark S SSR2 version 2.0), then the leaves were scanned (HP DeskJet GT S822) and their areas were measured using Image J v.1.44 software. To obtain the dry mass, the leaves were dried in an oven (315SE - FANEM) at 60°C until their mass became constant. Leaf longevity (measured in days), was evaluated in five pots which were not watered, allowing leaf permanence under drought conditions to be observed.

Phylogenetic signal analysis

To address whether phylogenetically closely related species are also functionally similar we used phylogenetic independent contrasts (PIC), that denote the existence of a phylogenetic signal. This signal can be strong, indicating phylogenetic conservatism, or weak, indicating no phylogenetic conservatism. We analyzed whether a phylogenetic signal was present in each of the traits measured. A phylogenetic signal is the tendency of taxonomically closer species to show more similar traits than expected at random (Blomberg; Garland, 2002). PIC consist of the difference between two species in the values of a given trait divided by the phylogenetic distance between them (Felsenstein 1985). Therefore, if two closely related species are very similar, the PIC value is low. To identify whether the PIC value is lower than expected at random, we used randomized positions of the species in the phylogenetic tree and determined a mean PIC, then we compared the observed PIC to the

simulated value, considering it significant, i.e., presence of the phylogenetic signal, if the P value is <0.025 (Münkemüller *et al.*, 2012). In addition, to identify whether there was phylogenetic conservatism of the traits, the strength of the phylogenetic signal was quantified using Blomberg's K (Blomberg *et al.*, 2003). If Blomberg's K is equal to one ($K = 1$) this indicates that the traits match Brownian motion expectation, K less than one ($K < 1$) indicates low phylogenetic signal (species more different than expected by chance), and K larger than one ($K > 1$) indicates a high phylogenetic signal (trait conservatism). Then, in order to test the significance of K , the values of the traits were randomized 999 times to generate a null distribution where the p value was calculated.

Relative interaction index

The nature (positive or negative) and intensity of the interactions were determined using the relative interaction index RII (Armas *et al.*, 2004). To calculate this index, the dry mass of the individuals was obtained at the end of the experiment (four months). For this, the shoot and root system were dried in an oven at 60 °C until reaching constant mass (72 hours), checked with a precision scale. The RII index is determined using the formula: $RII = (B_w - B_o) / (B_w + B_o)$, where B_w is the final biomass of the species growing in association and B_o is the final biomass of the species growing individually. When RII is negative (between 0 and -1), it means that the species are in competition and there is high niche overlap. On the other hand, if the RII value is positive (between 0 and +1), it is interpreted that the species are in facilitation and niche overlap is minimal. Finally, if the RII value is very close to 0 (between 0 and 0.05) the species are considered to have a neutral interaction and there is niche partitioning.

Data analysis

We verified that our data were normally distributed and homoscedastic and then we applied a t-test ($\alpha < 0.05$) to compare each trait in each pair of species (between species and within groups). An F-test ($\alpha < 0.05$) was used to compare sensitivity to transpiration (σ) and maximum transpiration rate per unit of hydraulic conductivity (Λ) between treatments.

To examine whether there was phylogenetic conservatism of the traits, the phylogenetic signal for continuous traits was quantified using Blomberg's K (Material and Methods, section 2.4).

Finally, to investigate whether phylogenetically closely related species compete more than distantly related species, a two-factor analysis of variance was performed by comparing the RII values of the phylogenetic relatedness between species (close, intermediate and distant) and interaction (interspecific and intraspecific). The Analysis of variance was preceded by the Shapiro-Wilk test $P > 0.05$ and homoscedasticity test $P > 0.05$, followed by the Tukey post-hoc test, $\alpha < 0.05$.

RESULTS

Functional traits and phylogenetic conservatism

There was a relationship between species functionality and phylogenetic gradient. The pair of species with greater phylogenetic proximity, *C. glazioviana* and *C. oconcalyx*, had four differences and seven similarities in functional traits, differing in only one morphological trait (LDM), one physiological trait (gs_{max}) and two hydraulic traits (Λ and πTLP ; Table 1). The species of the genus *Mimosa* (intermediate pair) differed in four morphological traits (SLA, SLM, LDM and LLD), one physiological trait (WUE) and one hydraulic trait (Λ ; Table 1), totaling six differences and five similarities. The pair composed of co-family species (distant pair) showed a higher divergence of functional traits compared to the other pairs, differing in eight traits, four morphological (SLA, SLM, SWC, LLD), three physiological (A_{max} , gs_{max} and WUE) and one hydraulic (σ ; Table 1), with only three similar traits.

Although the species with a higher degree of relatedness also had greater similarity between functional traits (Table 1), this relationship was not confirmed by the phylogenetic signal (Table 2). In the comparison among the 11 functional traits for the six species, there was no presence of phylogenetic signal for any of them ($P > 0.025$; Table 2). This result indicates that there is no phylogenetic conservatism in the functional traits analyzed, suggesting that they are less similar than expected in a random phylogeny (Table 2).

Nature of interactions

In the intraspecific treatment, the species of the genus *Cordia* showed a neutral interaction, with indices of 0.01 and 0.02 for *C. glazioviana* and *C. oconcalyx*, (Table 1).

Respectively (Figure 2A). In the interspecific interaction, there were differences between RII, demonstrating negative interaction in *C. glazioviana* (RII = -0.10) and positive interaction in *C. oconcalyx* (RII = 0.12; Figure 2A). In the pair of species of the genus *Mimosa*, *M. caesalpinifolia* demonstrated positive intraspecific interaction (RII = 0.09), and negative interspecific interaction with *M. tenuiflora* (RII = -0.19; Figure 2B). Unlike *M. caesalpinifolia*, *M. tenuiflora* demonstrated negative intraspecific interaction (RII = -0.14) and positive interspecific interaction (RII = 0.06, Figure 2B). The intraspecific interactions for the co-family species (*L. ferrea* and *P. bracteosa*) demonstrated a negative interaction for *L. ferrea* (RII = -0.06) and neutral for *P. bracteosa* (RII = 0.04; Figure 2C). In contrast, *L. ferrea* had a positive interspecific interaction (RII = 0.16) and *P. bracteosa* showed a neutral interaction (RII = 0.00; Figure 2C).

When we consider the RII value grouping the species into the relatedness pairs (close, intermediate and distant) it was found that the interspecific interaction within the distant pair was positive, different to the intermediate pair that showed the most negative interaction ($P = 0.022$, Figure 3). Besides this difference, the distant pair had a positive interspecific interaction but a negative intraspecific interaction ($P = 0.012$).

Table 1. Comparison of morphological, physiological and hydraulic functional traits for each pair of species with different degrees of relatedness: *C. glazioviana* and *C. oconcalyx* (closer), *M. tenuiflora* and *M. caesalpinifolia* (intermediate), and *L. ferrea* and *P. bracteosa* (more distant).

Trait	Units	<i>C. glazioviana</i>	<i>C. oconcalyx</i>	t	P	<i>M. tenuiflora</i>	<i>M. caesalpinifolia</i>	t	P	<i>L. ferrea</i>	<i>P. bracteosa</i>	t	P
SLA	mm ² mg ⁻¹	18.29(±1.1)	18.86(±0.3)	0.49	0.49	19.53(±0.8)	22.58(±1.0)	2.17	0.03	20.96(±0.3)	19.30(±0.5)	2.48	0.01
SLM	mg ⁻¹ mm ²	0.05(±0.0)	0.04(±0.0)	0.83	0.83	0.05(±0.0)	0.04(±0.0)	2.06	0.04	0.04(±0.0)	0.05(±0.0)	2.10	0.04
RWC	g g ⁻¹	1.19(±0.1)	1.58(±0.1)	1.94	0.06	1.69(±0.0)	1.64(±0.1)	0.29	0.77	0.86(±0.0)	1.52(±0.0)	7.09	0.00
LDM	mg	317.5(±30.0)	498.7(±23.7)	4.73	0.00	293.3(±13.2)	368.7(±25)	2.64	0.01	324.3(±30.2)	325.2(±25.6)	0.02	0.09
LLD	Days	25.280(±0.3)	24.00(±0.6)	1.84	0.07	26.96(±0.7)	20.50(±0.9)	5.28	0.00	12.48(±0.7)	42.32(±1.2)	20.27	0.00
<i>A</i> _{max}	μmol CO ₂ m ⁻² s ⁻¹	8.96(±0.3)	8.22(±0.2)	1.73	0.08	12.67(±0.3)	11.78(±0.4)	1.62	0.11	7.94(±0.24)	5.05(±0.3)	6.34	0.00
<i>g</i> _{max}	mol m ⁻² s ⁻¹	0.29(±0.0)	0.25 (±0.0)	2.73	0.00	0.41(±0.0)	0.45(±0.0)	1.17	0.24	0.27(±0.0)	0.15(±0.0)	8.59	0.00
WUE	μmol CO ₂ mol H ₂ O ⁻¹	31.07(± 0.9)	32.66(±1.4)	0.92	0.36	31.02(±1.1)	27.34(±1.3)	2.09	0.04	29.3(±0.7)	33.51(±1.7)	2.21	0.03
σ	MPa	0.70(±0.0)	0.81(±0.0)	1.10*	0.29	0.98(±0.0)	0.73(±0.1)	2.71*	1.10	0.61(±0.0)	0.84(±0.0)	6.8*	0.01
Λ	MPa	-2.05(±0.1)	-1.49(±0.1)	21.1*	0.00	-1.47(±0.1)	-0.74(±0.0)	12.9*	0.00	-2.41(±0.1)	-1.76(±0.1)	1.87*	0.46
πTLP	MPa	-2.72(±0.0)	-2.56(±0.0)	5.97	0.00	-2.45(±0.06)	-2.45(±0.03)	2.59	0.97	-2.62(±0.0)	-2.67(±0.0)	0.52	0.59

The functional traits analyzed were: Specific leaf area (SLA), specific leaf mass (SLM), relative water content (RWC), leaf dry mass (LDM), leaf longevity under drought (LLD), maximum photosynthetic rate (*A*_{max}), stomatal conductance (gs), intrinsic water use efficiency (WUE), relative sensitivity of transpiration (σ), maximum transpiration rate per unit of hydraulic conductivity (Λ), turgor loss point (πTLP). *Values marked with asterisk were tested through comparisons between lines using F test.

Table 2. Phylogenetic signal in 11 functional traits among three pairs of species with different degrees of relatedness: *C. glazioviana* and *C. oconcalyx*, *M. tenuiflora* and *M. caesalpinifolia*, and *L. ferrea* and *P. bracteosa*.

Trait	Units	Range	Z	K	P
SLA	mm ² mg ⁻¹	18.293 – 22.580	-0.229	0.707	0.304
SLM	mg ⁻¹ mm ²	0.043 – 0.053	-0.062	0.667	0.518
RWC	g g ⁻¹	0.868 – 1.761	-0.002	0.646	0.423
LDM	mg	293.250 – 498.733	-0.586	0.759	0.398
LLD	Days	12.48 – 42.32	2.068	0.422	0.985
<i>A</i> _{max}	μmol CO ₂ m ⁻² s ⁻¹	5.050 – 12.669	-1.229	1.004	0.152
<i>g</i> _{max}	mol m ⁻² s ⁻¹	0.159 – 0.458	-1.250	1.015	0.161
WUE	μmol CO ₂ mol H ₂ O ⁻¹	27.344 – 33.511	0.569	0.560	0.694
σ	MPa	0.61 – 0.98	1.284	0.487	0.869
Λ	MPa	-0.74 – -2.41	-0.501	0.744	0.339
πTLP	MPa	-2.030 – -2.720	-0.869	0.813	0.224

Range – Interval of variation in the values of functional traits, Z – phylogenetic independent contrast, K – intensity of phylogenetic signal and P – significance of the test (α<0.025).

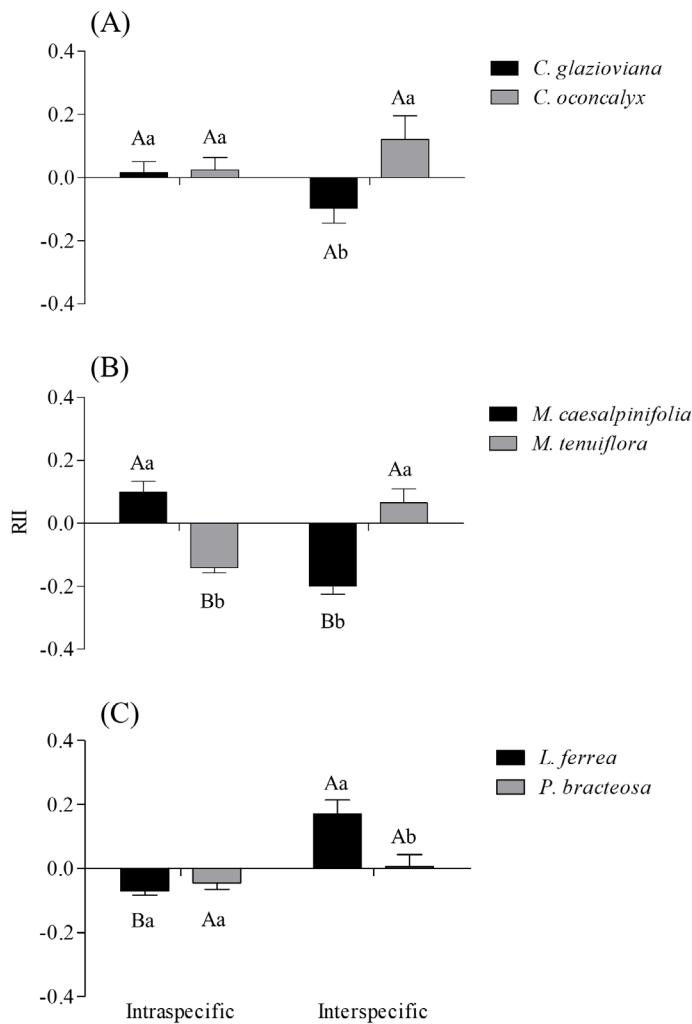


Figure 2. Relative interaction index (RII) and the respective confidence interval at 95% in intraspecific and interspecific interactions and in a phylogenetic relatedness gradient with closer, intermediate and distantly related pairs of species. The closely related pair is composed of *Cordia oncocalyx* and *C. glazioviana* (Boraginaceae), the distantly related pair by *Libidibia ferrea* and *Cenostigma bracteosa* (Caesalpinioidea-Fabaceae), and the intermediate pair by *Mimosa tenuiflora* and *M. caesalpinifolia* (Mimosoideae – Fabaceae). RII was calculated with the values of total biomass. Means (\pm S.D.) with different uppercase letters indicate significant differences between interactions within species, while lowercase letters represent the comparison between species within interactions.

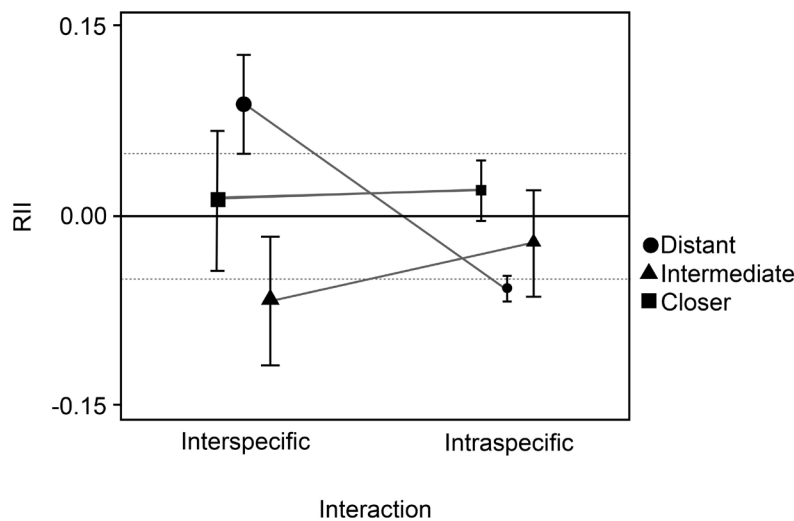


Figure 3. Relative interaction index (RII) and the respective confidence interval at 95% in intraspecific and interspecific interactions and in a phylogenetic relatedness gradient with close, intermediate and distantly related pairs of species. The closely related pair is composed of *Cordia oncocalyx* and *C. glazioviana* (Boraginaceae), the distantly related pair by *Libidibia ferrea* and *Cenostigma bracteosa* (Caesalpinioidea-Fabaceae) and intermediate pair by *Mimosa tenuiflora* and *M. caesalpinifolia* (Mimosoideae – Fabaceae). The horizontal black line represents the absolute neutral interaction and the dashed grey line represents the limits of the neutral interaction. Positive values indicate facilitation and negative values indicate competition. Means (\pm S.D.) with different lower-case letters indicate significant differences in phylogenetic relatedness within interspecific interaction, and upper-case letters indicate significant differences in interaction within distant pairs (Tukey test $P < 0.05$).

DISCUSSION

The competition-relatedness hypothesis (CRH) predicts that closer phylogenetically related species are more functionally similar and, by having similar niches, compete more than more distantly related species (Darwin, 1859). If phylogenetic proximity is equivalent to niche proximity, species need to preserve the functional traits of their ancestors as they evolve (Mayfield & Levine, 2010). However, studies that seek a phylogenetic signal in functional traits do not find phylogenetic conservatism of the ecological functions investigated (see Losos, 2008). The lack of conservation of functionality in the evolution of species is one of the main reasons that a positive relationship between increased competition and increased phylogenetic proximity is not frequently observed (e. g. Cahill *et al.*, 2007; Fritschie *et al.*, 2014; Alexandrou *et al.*, 2015). Our results demonstrate that the six species studied did not show a phylogenetic signal of functional traits, particularly those of water use strategies (A_{max} , $g_{s_{max}}$ and WUE). However, we provide evidence that there is greater functional similarity in the pair of phylogenetically more closely related species and that such similarity decreases with the increase in phylogenetic distance. The greater the phylogenetic distance, the more significant differences were found in the measurements of morphological, physiological and hydraulic variables of the species growing alone.

Although the competition-relatedness hypothesis (CRH) is currently of great interest, few studies support it (Alexandrou *et al.*, 2014). In a meta-analysis, Venail *et al.* (2014) surveyed 20 experimental studies and, of these, only six provided support for the CRH. In addition to this, to accept the CRH it is also necessary to accept that there is greater competition between more functionally similar species (MacArthur & Levins, 1967), which is not always found (Mayfield & Levine, 2010). It is necessary to consider that traits that confer competitive ability of a species could appear in the evolutionary history of a specific species, and not be conserved traits (Mayfield & Levine, 2010). In this study, the competitive ability of one species (*M. tenuiflora*) which has high vegetative growth (Figueiredo *et al.*, 2012) had an impact on the experiment, so that of the three species pairs studied, the pair that contained *M. tenuiflora* was the only one that did not corroborate the CRH.

In this study, we consider the intraspecific treatment to represent the highest degree of niche similarity. The closely (and more functionally similar), *C. glazioviana* and *C. oncocalyx* species pair showed the same result for both species whether in intraspecific or interspecific competition; i.e. the effect was the same whether the neighbouring plant was an individual of the same or a congeneric species. In the intermediate pair, it was possible to detect the competitive effect of *M. tenuiflora*. When this species grows with itself there is a negative impact on the individual of the same species, but when *M. tenuiflora* grows with an *M. caesalpinifolia* individual, the result is positive for the former and negative for the latter. Finally, the distant pair showed a

classic niche differentiation response. For this pair, when the species grew in intraspecific competition there was a negative effect, and when they grew with each other, *P. bracteosa* demonstrated a neutral interaction and *L. ferrea* showed a positive interaction. Although we are looking for simplifications of the complex reality, our results partly corroborate the CRH. Only the intermediate pair did not corroborate the CRH, because competition was observed for the closely phylogenetically related pair and facilitation was found for the distantly related pair. Although we found a pattern, one pair did not corroborate the CRH, meaning that other factors should be considered in the understanding of competition and coexistence among species.

Due to greater niche similarity, the intraspecific interactions were expected to have a more negative effect than the interspecific interactions, but the effect of this interaction was different depending on the species involved. For the congeneric species of the same clade, the intraspecific interaction was neutral, for the congeneric species of distinct clades, this interaction was competitive for *M. tenuiflora* and facilitative for *M. caesalpinifolia*, and for the species of the same family the result was competition for both species. Once again, we note the strong competitive ability of *M. tenuiflora*, and that *M. caesalpinifolia*, a nitrogen-fixing species (Barros *et al.*, 2018), showed a facilitative interaction in the intraspecific treatment. These results, once again, demonstrate the importance of intrinsic traits of the species involved in the interaction.

What we can synthesize from all these interactions is that facilitation is found in phylogenetically distant species and that competition is not always related to phylogenetic distance. Phylogenetically more distant species are more likely to interact positively, through facilitation (Valiente-Banuet and Verdú, 2007). Niche partition and facilitation were found in the pair of greater phylogenetic distance, and these results corroborate those reported in other studies (Verdú *et al.*, 2009; Castillo, Verdu & Valiente-Banuet, 2010; Duarte *et al.* 2021). Facilitation, a positive interaction, usually affects the germination and establishment of coexisting species through the improvement of microenvironmental conditions and/or resource availability (Gómez-Aparicio *et al.*, 2004). The set of conditions required by a species at the time of germination and initial establishment is called the regeneration niche (Grubb, 1977). According to Valiente-Banuet and Verdú (2007), regeneration niche traits are conserved throughout evolution, i.e., similar species require similar conditions to germinate and establish themselves and, therefore, phylogenetically distant species grow under the canopy of nurse species, and not of similar species and much less of the same species. Facilitation between distant species can be easily observed when studying shrub species facilitating cactus species (e.g., Valiente-Banuet *et al.*, 1991; Drezner, 2006; Flores-Torres *et al.*, 2019). Although in our work we studied species with the same growth form and with a certain phylogenetic relatedness, facilitation was also

found in species of the same family (Fabaceae, the more distantly related pair).

We were expecting that, if the functional traits were conserved and the environmental filter (water seasonality) selected the species in the semiarid region, then the phylogenetically close species would coexist and that competition would be less significant (see Webb *et al.*, 2002). However, a negative interaction was found in two of the three pairs of species. We cannot fail to point out that our study was conducted in 8 L pots, each containing two individuals, and therefore negative interaction was stimulated due to space limitations. Other studies evaluating competition and facilitation between species in a gradient of phylogenetic relatedness should be conducted under field conditions to get closer to reality.

In conclusion, phylogenetic distance alone cannot be used to determine the interaction results in species associations, and the competitive ability of each species should also be considered when evaluating species coexistence. The coexistence mechanisms can be used to predict biological invasions and to determine better species combinations for the restoration and recovery of degraded areas.

AUTHORSHIP CONTRIBUTION

Project Idea: BCS, BSM, FSA, RBZ.

Funding: FSA, RBZ;

Database: MTT, BCS, BSM.

Processing: MTT, BCS.

Analysis: MTT, BCS, BSM.

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