Could biological invasion by *Cryptostegia madagascariensis* alter the composition of the arbuscular mycorrhizal fungal community in semi-arid Brazil?

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ABSTRACT

Biological invasions pose a serious threat to native semi-arid areas of Brazil, especially in areas of the state of Ceará that are typically invaded by *Cryptostegia madagascariensis*, an exotic plant species from Madagascar. However, how this biological invasion influences the composition of the arbuscular mycorrhizal fungal (AMF) community and how this affects further invasion by *C. madagascariensis* is not well known. Here we tested how inoculation with species of AMF affects the development of this invasive plant. We analyzed and compared the AMF community composition of four different stages of biological invasion by *C. madagascariensis*, and examined the effects of inoculation with these four AMF communities, plus a dominant AMF species (*Rhizoglomus intraradices*) on plant dry biomass, root colonization, plant phosphorous concentration, and plant responsiveness to mycorrhizas of plants of *C. madagascariensis*. We found that all studied treatments (except the inoculum from the native plant root zone) promoted the growth of *C. madagascariensis* and lead to a higher P concentration. Our results demonstrate that the invader might be altering the composition of the AMF community in field conditions, because inoculation with this community enhanced invader growth, root colonization, and P uptake.

Keywords: AMF inoculation, biological invasion, drylands, Glomeromycota, *Rhizoglomus intraradices*.

Introduction

To understand more about the effects of invasion on arbuscular mycorrhizal fungi (AMF) community, current research has focused on feedback between AMF communities and invasive species (Zhang *et al.* 2010), in the host-fungal pairing (Klironomos 2003), and how AMF can impact the performance of invasive species (Stampe & Daehler 2003). This work is the first field study to examine the impacts of *C. madagascariensis* on AMF community composition along an invasion gradient and to investigate how the changed-AMF community may contribute to create a "window of opportunity" (Johnstone 1986; Agrawal *et al.* 2005) and consequently influencing plant invasions (Blumenthal 2005; Kulmatiski & Kardol 2008).

Plant-AMF specificity affects plant invasion (Mummey & Rillig 2006; Parkash & Aggarwal 2009; Zhang *et al.* 2010) and may in some cases strengthen invasive ability of plants through complex feedback loops (Richardson *et al.* 2000). Plants provide AMF with energy while AMF provides water and nutrients such as phosphorus and nitrogen to the plant host (Smith & Read 2008; Hodge & Storer 2014). Plants have affected the AMF community structure through influences on soil resource availability, like available P (Alguacil *et al.* 2012; Ji *et al.* 2012; Beauregard *et al.* 2013; Lekberg *et al.* 2013; Geel *et al.* 2015;

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Tancredo Augusto Feitosa de Souza, Susana Rodriguez–Echeverría,
Leonaldo Alves de Andrade and Helena Freitas

Wang *et al.* 2015), and these microorganisms influence plant community composition and ecosystem function (Heijden *et al.* 2008).

AMF benefit plants by mediating the availability of important plant nutrients (Smith & Read 2008; Hodge & Storer 2014)**,** promote plant growth and offer protection against drought and soil pathogens (Rodríguez-Echeverría *et al.* 2009; Grümberg *et al.* 2015; Ortiz *et al.* 2015). We examined the AMF community from *Cryptostegia madagascariensis* root zone, and compared it with the AMF community from *Copernicia prunifera* root zone.

In Ceará State, Brazil, the exotic species *C. madagascariensis* occupies dense areas, occurring widespread and destructive form on native vegetation, particularly *C. prunifera*, because the exotic species can strangle and kill the palm tree by climbing over it and completely eliminating access to light (Sousa *et al.* 2013), suggesting that this native plant may be competitively affected by *C. madagascariensis* in the field conditions.

Cryptostegia madagascariensis is a woody-perennial vine that is native to south-west Madagascar. It has been introduced in many Brazilian States by man because of its attractive flowers (Silva *et al.* 2008). This invasive plant forms dense aboveground stands, suggesting that it exerts a large impact on the AMF community. We studied this alien species into *Copernicia* forest areas ("Matas de Carnaúba") in Ceará State, Brazil where this species is expanding its range, and the edge of this range is a dangerous invasion front.

Copernicia palm is highly important to local economy of Ceará State, popularly known as the "Tree of life" used in a wide variety of applications (civil construction, craftwork, animal feed and pharmaceutical industry) (DS Pereira *et al.* 2014). Many programs of development of seedling production have been done, encouragement of tree planting and preservation of *Copernicia* forest areas have become important; however, *C. madagascariensis* is capable of invading this environment, thus, threatening native endemic biodiversity.

Thus, we designed a sampling strategy to study how *C. madagascariensis* invasion alters arbuscular mycorrhizal fungi community composition of the Copernicia Forest, Ceará State, Brazil and hypothesized that (1) *C. madagascariensis* invasion alters AMF community composition and soil properties, like soil pH and total organic carbon in the Copernicia Forest; (2) Changed-AMF community can promote the invasive growth; and (3) *C. madagascariensis* plants can experience a strong relationship with dominant AMF species (*Rhizoglomus intraradices* in our case). We based these hypotheses on (a) enhanced mutualisms hypothesis proposed by Richardson *et al.* (2000) and Reinhart & Callaway (2006); (b) the invasion opportunity windows hypothesis proposed by Johnstone (1986) and Agrawal *et al.* (2005); (c) the resource-enemy release hypothesis proposed by Blumenthal (2005); and (d) the disturbance-contingent niche creation model proposed by Kulmatiski & Kardol (2008).

Materials and Methods

Site description and sampling methods

We selected study areas near Ibaretama, Ceará, Brazil (06°51'11.3"S, 35°55'51.5"W, and average altitude 109 m). The climatic conditions of studied areas is Bsh (Köppen), hot semi-arid with hot summers and mild to warm winters, annual precipitation and temperature of 600 mm and 30 °C respectively. Rainfall in these sites is scanty, unpredictable and irregular (Alves *et al.* 2009). The soil type was classified as an Eutric Vertisol (WRB 2006).

The invasive *Cryptostegia madagascariensis* Bojer ex. Decne. and the native *Copernicia prunifera* (Mill.) H. E. Moore, which co-occur in the "Copernicia forest" in the Brazilian xeric shrubland from Ceará State, Brazil, were selected. The seeds of the invader were collected directly from the studied areas.

We collected samples from the root zone of both plants in four different stages of biological invasion by *C. madagascariensis*: Exotic - areas that contains only *C. madagascariensis* plants (We collected samples from root zone of young plants with an average height between 0.5 and 0.9 m); Native – areas that contains only *C. prunifera* plants (We collected samples from root zone of plants with an estimated average height of 12.0 m); Invasive Stage 1 (Invasive1) - areas that contains *C. madagascariensis* plants (We collected samples from root zone of plants with an average height between 1.5 and 3.0 m) climbing over *C. prunifera* plants with an estimated average height of 10.0 m; and Invasive Stage 2 (Invasive2) – areas that contains *C. madagascariensis* plants (We collected samples from root zone of plants with undetermined average height) climbing over dead *C. prunifera* plants with an estimated average height of 8.0 m).

We established for each stage of biological invasion (four stages – Native, Exotic, Invasive1, Invasive2) by *C. madagascariensis* forty plots of 100 m^2 according to Fortin & Dale (2005). We collected samples from forty plants in each plot that were pooled within each plot. We selected plants with the following characteristics: plant with diameter near soil surface > 3 cm and without any individual from a different plant species at a distance lower than 3 m to the sampling point (Daubenmire 1968; Fortin & Dale 2005; Caifa & Martins 2007; Costa & Araújo 2007; Durigan 2009).

Root zone samples (including soil and root fragments) were collected out near the drip line and beyond (0-20 cm deep), at the beginning of September 2012 during the dry period. The root zone samples from each species in each plot were bulked, mixed and stored at 4 °C until host-plant

bioassays. Thus, we analyzed 40 composite samples for each invasion stage (N=160, 40 composite samples x four stages). The sampling period was done during the dry season because fungal sporulation is expected to be higher at this time in semi-arid environments (Silva *et al.* 2014). The root zone samples collected from the field was divided into portions intended for: chemical soil characterization, AMF community assessment, and mounting trap cultures.

For chemical characterization we analyzed soil pH, total organic carbon, total nitrogen and available phosphorus from field soil. Soil pH was measured in a suspension of soil and distilled water (1:2.5 v:v, soil: water suspension) (Black 1965). Total organic carbon was estimated according methodology described by Okalebo *et al.* (1993). For nitrogen, the soil samples were digested and total nitrogen was quantified according to Kjeldahl (Black 1965). Available phosphorus (Olsen's P) was determined colorimetrically on spectrophotometer at 882 nm by extraction with sodium bicarbonate for 30 min (Olsen *et al.* 1954).

AMF communities were selected from each studied biological invasion stage. These communities naturally exist in the root zone of both studied model plants, where we classified as a changed-AMF community the community of arbuscular mycorrhizal fungi from Exotic, Invasive1 and Invasive2 areas; and as an unaltered-AMF community the community of AMF from Native areas. So, a trap culture of each AMF community for each condition was established from field soil (500 g) in plastic pots (2 kg).

The AMF communities of trap cultures were propagated on a common host-plant (*Zea mays* L.). This species is a standard host-plant used for mycorrhiza trap cultures and inoculum potential assays according to INVAM (http://invam.caf.wvu.edu/). The plants were grown in sterilized sand: field soil (3:1 m:m) in a greenhouse condition at University of Coimbra for four months until sporulation. The host-plants received weekly amounts of a modified nutrient solution (Hoagland & Arnon 1939) containing (in mg L^{-1}): 554.0 KCl, 200.0 NaH$_2$PO$_4$. H$_2$O, 2.24 MgSO$_4$, 520.0 CaCl$_2$.H$_2$O, 1.7 MnSO$_4$, 0.25 CuSO$_4$.5H$_2$O, 0.30 ZnSO$_4$.7H$_2$0, 5.0 NaCl, 3.0 H$_3$O$_3$, 0.09 (NH$_4$)6Mo$_7$O$_{24}$.4H$_2$O, and 32.9 Na-Fe EDTA. A portion of soil (500 g) from each studied condition was then used to create the initial AMF-inoculum (The soil, roots, and spores were used as inoculum) used for the experiment with *C. madagascariensis* plants. Another portion of soil (100 g) was taken from each studied condition for spore extraction and subsequent identification of AMF species for evaluation of the ecological index. The average temperature in the greenhouse was 28 °C, ranging from 20 °C to 35 °C, an irradiance of up to 70 % of full sun, relative humidity ranging from 65 to 75 %, and photoperiod of 16:8 h L:D.

Spores were extracted by the wet sieving technique (Gerdemann & Nicolson 1963) followed by sucrose centrifugation (Jenkins 1964). Initially, extracted spores were examined in water under a dissecting microscope. They were separated based on morphological characteristics. After it, they were mounted in polyvinyl alcohol lacto-glycerol (PVLG) with or without addition of Melzer's reagent (Walker *et al.* 2007). The identification of species was based on the descriptions provided by Schenck & Perez (1990), publications with descriptions of new genera and families (i.e. Oehl *et al.* 2008), and by consulting the international culture collection of arbuscular mycorrhizal fungi database – INVAM (http://invam.caf.wvu.edu). In this work we adopted the classification proposed by Oehl *et al.* (2011), including recently described new taxa (i.e. Goto *et al.* 2012 and Sieverding *et al.* 2014). After AMF species identification, provide directly from field, we calculated the following ecological index: diversity index (H) proposed by Shannon & Weaver (1949), and dominance index (C) proposed by Simpson (1949) for each studied AMF community. In addition, we assessed spore abundance (total number of spores), spore abundance of AMF species (number of spores of each AMF species recorded), and the species occurrence frequency (FO_i) of each AMF species. FO_i was calculated using the following equation: $FO_i = n_i/N$, where n_i is the number of times an AMF species was observed and N is the total of AMF spores observed from each stage of invasion. We classify the results of FO_i based on the Zhang's frequency of occurrence classification: dominant ($FO_i > 50\%$), most common ($31 \leq FO_i \leq 50\%$), common ($10 \leq FO_i \leq 30\%$) and rare ($FO_i < 10\%$) (Zhang *et al.* 2004). The AMF species were also classified as generalists (present in all areas), intermediate (present in three or two areas) or exclusive (present in only one area) (Stürmer & Siqueira 2011).

The bioassay experiments involved a completely randomized design with six treatments: non-inoculation (Control) and five inoculation treatments with inoculum of AMF communities from: Native = Inoculum from *C. prunifera* areas; Exotic = Inoculum from *C. madagascariensis* areas; Invasive1 = Inoculum from Invasive Stage 1 areas (beginning of invasion); Invasive2 = Inoculum from Invasive Stage 2 areas (invasion successful); and inoculum composed by *Rhizoglomus intraradices* (N.C. Schenck & G.S. Sm.) Sieverd. G.A. Silva & Oehl (Rhi), which we found as a dominant AMF species into changed-AMF communities from Exotic, Invasive1, and Invasive2 areas. The control received 500 ml of filtrate from 500 g of AMF-inoculum with no mycorrhizal spores obtained by sieving through a 25 μm mesh, and 500 g of sterilized mixed AMF-inoculum (a mix of all AMF-inoculums by model plant), thereby controlling for potential mineral and non-mycorrhizal microbial components of the AMF-inoculum. Each treatment had 30 replicates that were coming from 30 independent replicates at trap culture stage.

The experiment was also conducted in a greenhouse at University of Coimbra with average temperatures of 25/16 °C (day/night). Seeds of *C. madagascariensis* were surface

Tancredo Augusto Feitosa de Souza, Susana Rodriguez–Echeverría,
Leonaldo Alves de Andrade and Helena Freitas

sterilized in 10% sodium hypochlorite for 10 minutes, and then thoroughly rinsed with deionized water. Seeds were then were germinated in trays containing autoclaved sand (Twice at 121 °C for twenty minutes in two consecutive days). Seven days after emergence, seedlings (plant height varying 2-4 cm) were selected and individually transferred to plastics pots containing 2000 g of substrate (1500 g of autoclaved sand mixed with 500 g of soil containing AMF-inoculum, with the number of infective propagules per gram of soil ranging between 5.35 and 6.18. The number of infective propagules was determined following the protocol described by Habte & Osorio (2001). All pots were covered with aluminum wrap around the seedling to prevent dehydration and external contamination, and the plants were watered with tap water as necessary and fertilized with the same nutrient solution used on trap culture (Hoagland & Arnon 1939).

Five months after planting, the plants were harvested. Roots were separated from shoots, and immediately the fresh roots were weighed. A total of 0.5 g of each fresh root sample was used for determination of root colonization. The remaining material and shoots were oven-dried (72°C for 48h) and used for measurement of shoot-, and root dry biomass. The amount of colonization was estimated using a grid line intersect method with examination of 100 intersects under a compound microscope at 200X magnification (Phillips & Hayman 1970; Giovannetti & Mosse 1980).

The plant responsiveness to mycorrhizas (PRM) was calculated using dry biomass of plants in the AMF-inoculum treatment and the control (Janos *et al.* 2007), using the formula: PRM = $[(B_{AMF}-B_{control})/B_{AMF}]$ x 100, where B_{AMF} is dry biomass of model plants in the AMF-inoculum treatment and $B_{control}$ is dry biomass of the control treatment.

A known mass of the grounded material was digested in a digestion flask containing a triple acid mixture [HNO_3:H_2SO_4: HCl (60%), with a ratio of 10:1:4, respectively] to analyze the total P. Phosphorous was analyzed using the vanadate molybdate colorimetric method (Jackson 1973). We also calculated the AMF inoculum effect on plant P content (IEP), using the formula: IEP = $(P_{AMF} - P_{Control})/(P_{AMF} + P_{Control})$, where P_{AMF} is plant P content in AMF inoculum treatment (adapted from Armas *et al.* 2005), assuring that the AMF inoculum effect can only range from -1 to +1, with positive values indicating positive effects of AMF on plant P content, and negative values indicating negative effects.

The relationships between the AMF community structure and soil properties were examined using correlation analyses by the Pearson correlation coefficient (*r*). One-way ANOVA was used to test the effect of AMF inoculum on plant dry biomass, and plant P content for *C. madagascariensis* plants. Data sets were transformed (arcsin square root for percentage variables and logarithmic for other variables), but the results were presented in their original

scale of measurement (means with stander deviation) (Zar 1984). For mycorrhizal colonization, PRM and IEP, the Control treatments were excluded from the analyses since the results were null, and were analyzed using Tukey's HSD *post-hoc* comparison test (p<0.05). Significant differences among AMF inoculum treatment on plant dry biomass and plant P content were also determined using the Tukey's HSD *post-hoc* comparison test (p<0.05). One way ANOVA and correlation analyses were conducted using SAS 9.1.3 Portable and the ecological indexes were conducted using MVSP 3.1 (Kovach 2007).

Results

AMF community and soil properties from field samples

The samples from *Exotic* and *Native* areas had the largest number of AMF identified species, with 16.80 and 16.00 identified species respectively, followed by Invasive1 (11.70) and Invasive2 (8.50). The number of spores was 2.74 spores/g soil in Exotic; 5.17 spores/g soil in Native; 1.16 spores/g soil in Invasive1; and 0.86 spores/g soil in Invasive2. Diversity (H, Shannon's index) and dominance (C, Simpson's index) in the Exotic and Native was about 2.42 and 0.89 respectively, but in the Invasive1 and Invasive2 these values changed to 1.76 and 0.76 respectively (Tab. 1).

Including all studied root zones, 19 different AMF species were identified belonging to eleven genera – *Acaulospora* (2), *Claroideoglomus* (2), *Dentiscutata* (2), *Entrophospora* (1), *Funneliformis* (2), *Gigaspora* (3), *Glomus* (1), *Quatunica* (1), *Racocetra* (1), *Rhizoglomus* (3) and *Scutellospora* (1) (Tab. 2). Of the total AMF species, 12 (63.15%) were classified as intermediate species: *Acaulospora denticulata, A. tuberculata, Claroideoglomus claroideum, Dentiscutata cerradensis, D. heterograma, Entrophospora infrequens, Gigaspora albida, G. decipiens, G. gigantea, Quatunica erythropus, Rhizoglomus intraradices,* and *Scutellospora calospora*; and 7 (36.85%) were classified as generalist: *Claroideoglomus etunicatum, Funneliformis caledonium, F. geosporum, Glomus multicaule, Racocetra coralloidea, Rhizoglomus aggregatum,* and *Rhizoglomus clarum*. We did not found any AMF species with frequency of occurrence classified as rare (*sensu* Zhang *et al.* 2004), or exclusive (*sensu* Stürmer & Siqueira 2011) in our study (Tab. 2).

In the *Exotic* root zone, four AMF species (21.05%) were classified as Common: *C. etunicatum, D. heterograma, F. caledonium,* and *R. clarum;* and 15 AMF species (78.95%) were classified as Rare: *A. denticulata, A. tuberculata, C. claroideum, D. cerradensis, D. erythropus, E. infrequens, F. geosporum, G. albida, G. decipiens, G. gigantea, R. aggregatum, R. multicaule, R. coralloidea, R. intraradices,* and *S. calospora*. For the *Native* root zone, five AMF species (29.41%) were classified as Common: *C. etunicatum,*

Could biological invasion by *Cryptostegia madagascariensis* alter the composition of the arbuscular mycorrhizal fungal community in semi-arid Brazil?

Table 1. Mean of AMF identified species (IS), number of spores (NS), diversity (H) and dominance index (C) of each site (mean ± SD, N= 40) for each analyzed parameter.

Parameter	Exotic	Native	Invasive1	Invasive2	LSD
IS	16.80 ± 0.80	16.00 ± 0.90	11.70 ± 1.11	8.50 ± 1.93	1.13
NS (g soil^{-1})	2.74 ± 0.11	5.17 ± 1.65	1.16 ± 0.06	0.86 ± 0.08	0.59
H	2.41 ± 0.08	2.42 ± 0.07	2.14 ± 0.11	1.76 ± 0.08	0.04
C	0.88 ± 0.01	0.89 ± 0.01	0.85 ± 0.02	0.76 ± 0.02	0.05

Table 2. Frequency of occurrence (FO_i) of AMF species of each studied rhizosphere soil

AMF species	FO^1 (classification^2)				Species classification[3]
	Exotic	Native	Invasive1	Invasive2	
Order Diversisporales					
Family Acaulosporaceae					
Acaulospora denticulata Sieverd. & S. Toro	2.9 (R)	3.3 (R)	3.0 (R)	-	Intermediate
Acaulospora tuberculata Janos & Trappe	1.5 (R)	1.6 (R)	0.8 (R)	-	Intermediate
Order Gigasporales					
Family Dentiscutataceae					
Dentiscutata cerradensis (Spain & J. Miranda) Sieverd., F.A. Souza & Oehl	1.3 (R)	2.5 (R)	1.1 (R)	-	Intermediate
Dentiscutata heterogama (T.H. Nicolson & Gerd.) Sieverd., F.A. Souza & Oehl	10.2 (C)	10.8 (C)	1.5 (R)	-	Intermediate
Quatunica erythropus (Koske & C. Walker) F.A. Souza, Sieverd. & Oehl	1.8 (R)	1.4 (R)	0.4 (R)	-	Intermediate
Family Gigasporaceae					
Gigaspora albida N.C. Schenck & G.S. Sm.	0.6 (R)	1.2 (R)	-	-	Intermediate
Gigaspora decipiens I.R. Hall & L.K. Abbott	1.1 (R)	1.6 (R)	-	-	Intermediate
Gigaspora gigantea (T.H. Nicolson & Gerd.) Gerd. & Trappe	1.1 (R)	1.7 (R)	-	-	Intermediate
Family Racocetraceae					
Racocetra coralloidea (Trappe, Gerd. & I. Ho) Oehl, F.A. Souza & Sieverd.	2.1 (R)	4.1 (R)	4.9 (R)	5.9 (R)	Generalist
Family Scutellosporaceae					
Scutellospora calospora (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders	1.7 (R)	2.4 (R)	-	-	Intermediate
Order Glomerales					
Family Entrophosporaceae					
Entrophospora infrequens (I.R. Hall) R.N. Ames & R.W. Schneider	1.7 (R)	1.7 (R)	-	-	Intermediate
Claroideoglomus claroideum (N.C. Schenck & G.S. Sm.) C. Walker & Schüßler	2.4 (R)	-	8.9 (R)	6.9 (R)	Intermediate
Claroideoglomus etunicatum (W.C. Becker & Gerd.) C. Walker & Schüßler	15.5 (C)	16.0 (C)	14.9 (C)	5.2 (R)	Generalist
Family Glomeraceae					
Funneliformis caledonium (T.H. Nicolson & Gerd.) C. Walker & Schüßler	14.2 (C)	14.5 (C)	9.5 (R)	6.9 (R)	Generalist
Funneliformis geosporum (T.H. Nicolson & Gerd.) C. Walker & Schüßler	10.0 (R)	10.1 (C)	7.7 (R)	4.5 (R)	Generalist
Glomus multicaule Gerdemann & B.K. Bakshi	6.3 (R)	6.6 (R)	2.8 (R)	2.6 (R)	Generalist
Rhizoglomus aggregatum (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl	3.5 (R)	5.1 (R)	5.8 (R)	7.9 (R)	Generalist
Rhizoglomus clarum (T.H. Nicolson & N.C. Schenck) Sieverd., G.A. Silva & Oehl	15.5 (C)	15.5 (C)	11.1 (C)	7.5 (R)	Generalist
Rhizoglomus intraradices (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl	6.4 (R)	-	27.7 (C)	52.7 (D)	Intermediate

^1FO_i = n_i/N, where n_i is the number of times an AMF species was observed and N is the total of AMF spores observed from each studied condition; ^{2}Classification of AMF frequency of occurrence proposed by Zhang et al. (2004): R – rare (FO < 10%); C – common (10 ≤ FO ≤ 30 %); MC – most common (31 ≤ FO ≤ 50); and D – dominant (FO > 50 %); ^{3}Species classification proposed by Stürmer and Siqueira (2011): generalists (present in all areas), intermediate (present in 2 or 3 areas) or exclusive (present in only one area).

F. caledonium, D. heterograma, F. geosporum, and *R. clarum;* and 12 AMF species (70.59%) were classified as Rare: *A. denticulata, A. tuberculata, D. cerradensis, E. infrequens, G. albida, G. decipiens, G. gigantea, G. multicaule, Q. erythropus, R. aggregatum, R. coralloidea,* and *S. calospora* (Tab. 2).

In the Invasive1, three AMF species (21.42%) were classified as Common: *C. etunicatum, R. clarum* and *R. intraradices;* and 11 AMF species (78.58%) were classified as Rare: *A. denticulata, A. tuberculata, C. claroideum, Dentiscutata cerradensis, D. heterograma, F. caledonium, F. geosporum, G. multicaule, Q. erythropus, R. aggregatum,* and *R. coralloidea. R. intraradices* was the only dominant AMF species (11.2%) recorded in the Invasive2. Eight AMF species were classified as Rare: *C. claroideum, C. etunicatum, F. caledonium, F. geosporum, G. multicaule, R. aggregatum, R. coralloidea* and *R. clarum* (Tab. 2).

Soil pH, total organic carbon, total nitrogen and available phosphorous were higher in the Invasive2 than other studied root zones, but no significant differences were observed between Exotic and Native root zones for any of the soil properties analyzed (Tab. 3).

Bioassay

The AMF inoculation effect on *C. madagascariensis* plant dry biomass varied among the AMF inoculation treatment ($F_{5, 179} = 8.74, p<0.0001$). Native had no differences with control on plant dry biomass, whereas Rhi, Invasive2, Invasive1 and Exotic had a significant positive effect (Tukey's post-hoc test, p<0.05). The plant dry biomass decreased in the following order: Rhi > Invasive2 > Invasive1 = Exotic > Control = Native (Fig. 1).

There were significant effects of AMF inoculation on the root colonization ($F_{4, 149} = 89.67, p<0.0001$). No differences were found between Rhi, Invasive2, 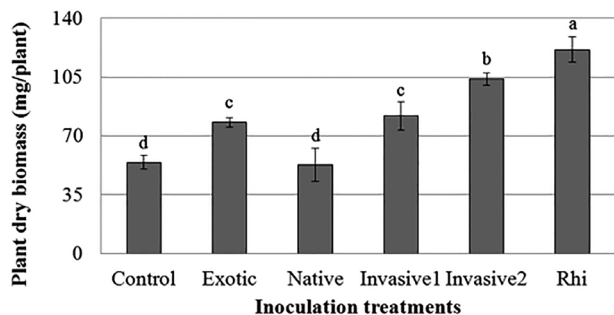Invasive1, and Exotic treatments, but when compared with the Native results, these treatments significantly enhanced the root colonization of *C. madagascariensis* plants. We did not found any AMF colonization structure inside of roots from control, so we excluded it from the statistical analyses (Fig. 2).

The AMF inoculation significantly influenced plant P content ($F_{5, 179} = 13.62, p<0.0001$). The inoculation with AMF community from Native root zone had lower plant P content than other five treatments. *C. madagascariensis* plants had more plant P content when growing with AMF inoculum from Invasive2 root zone, but the exotic plant also experienced positive effects growing with Rhi, Invasive1, and Exotic (Fig. 3).

Differences in AMF inoculation treatments were also detected in PRM ($F_{4, 149} = 4.59, p<0.001$) and IEP ($F_{4, 149} = 4.59, p<0.001$). Plant growth responses (in terms of the plant responsiveness to mycorrhizas and inoculum effect on P uptake) of the *C. madagascariensis* plants to AMF
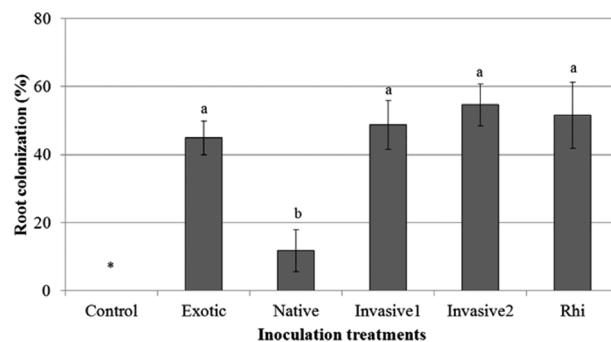
Figure 2. Root colonization (%) in each inoculation treatment (mean ± SE, N=30), Exotic = Inoculation with AMF from *C. madagascariensis* root zone; Native = Inoculation with AMF from *C. prunifera* root zone; Invasive1 = Inoculation with AMF from Invasive1 root zone; Invasive2 = Inoculation with AMF from Invasive2 root zone; Rhi = Inoculation with *Rhizoglomus intraradices*. Different letters mean significant differences (Tukey's HSD *post hoc* tests after one-way ANOVA).

Figure 1. Plant dry biomass (mg plant⁻¹) in each inoculation treatment (mean ± SE, N=30), Control = non-inoculation; Exotic = Inoculation with AMF from *C. madagascariensis* root zone; Native = Inoculation with AMF from *C. prunifera* root zone; Invasive1 = Inoculation with AMF from Invasive1 root zone; Invasive2 = Inoculation with AMF from Invasive2 root zone; Rhi = Inoculation with *Rhizoglomus intraradices*. Different letters mean significant differences (Tukey's HSD *post hoc* tests after one-way ANOVA).
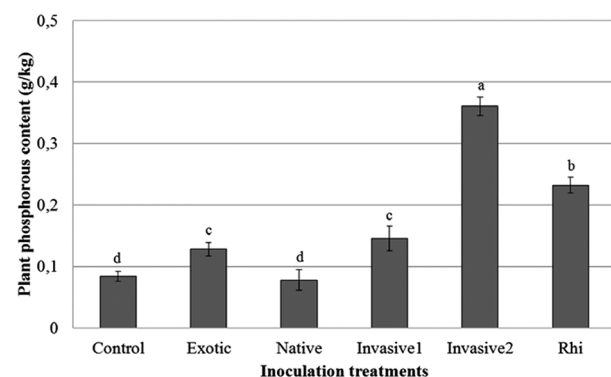
Figure 3. Plant phosphorus content (mg kg⁻¹) in each inoculation treatment (mean ± SE, N=30), Control = non-inoculation; Exotic = Inoculation with AMF from *C. madagascariensis* root zone; Native = Inoculation with AMF from *C. prunifera* root zone; Invasive1 = Inoculation with AMF from Invasive1 root zone; Invasive2 = Inoculation with AMF from Invasive2 root zone; Rhi = Inoculation with *Rhizoglomus intraradices*. Different letters mean significant differences (Tukey's HSD *post hoc* tests after one-way ANOVA).

inoculation differed among the five treatments and both variables were lowest with AMF from the native root zone (Figs. 4-5). Analyzing the effects of other AMF inoculation treatments we found positive effects on PRM and EIP of *C. madagascariensis* plants growing in the Rhi, Invasive2, Invasive1 and Exotic inoculation treatments.

Discussion

The study of field samples shows that the biological invasion by *C. madagascariensis* changes AMF community composition (Tabs. 1-2) and soil properties (Tab. 3) along the different studied stages of biological invasion in the Brazilian semi-arid, supporting our first hypothesis. The results obtained in the bioassays demonstrate that these altered AMF communities, and especially *R. intraradices*, the most abundant species in the invaded area, promote the growth of the invasive *C. madagascariensis*, supporting the remaining original hypothesis of this study.
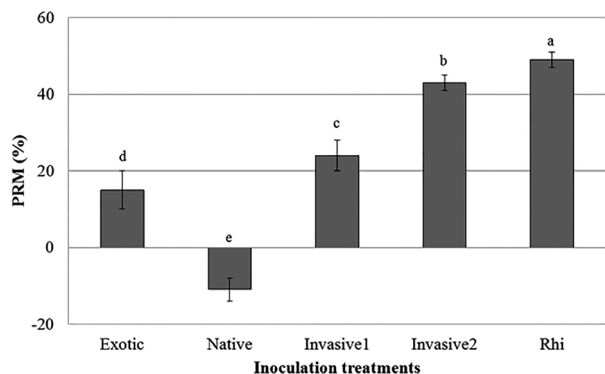

Figure 4. Plant responsiveness to mycorrhiza (PRM %) in each inoculation treatment (mean ± SE, N=30), Exotic = Inoculation with AMF from *C. madagascariensis* root zone; Native = Inoculation with AMF from *C. prunifera* root zone; Invasive1 = Inoculation with AMF from Invasive1 root zone; Invasive2 = Inoculation with AMF from Invasive2 root zone; Rhi = Inoculation with *Rhizoglomus intraradices*. Different letters mean significant differences (Tukey's HSD *post hoc* tests after one-way ANOVA).
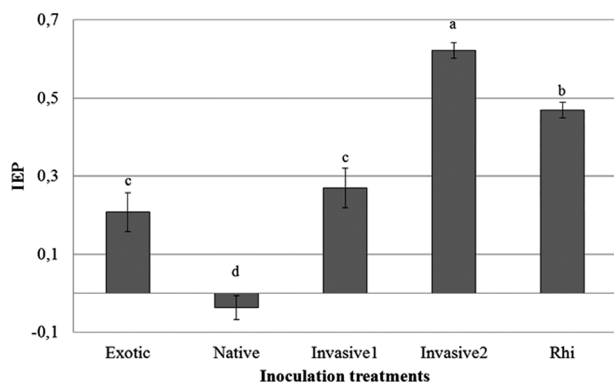

Figure 5. IEP in each inoculation treatment (mean ± SE, N=30), Exotic = Inoculation with AMF from *C. madagascariensis* root zone; Native = Inoculation with AMF from *C. prunifera* root zone; Invasive1 = Inoculation with AMF from Invasive1 root zone; Invasive2 = Inoculation with AMF from Invasive2 root zone; Rhi = Inoculation with *Rhizoglomus intraradices*. Different letters mean significant differences (Tukey's HSD *post hoc* tests after one-way ANOVA).

Table 3. Soil properties of the area of each invasion stage by *C. madagascariensis* (mean ± SD, N= 40) for each analyzed parameter.

Parameter	Exotic	Native	Invasive1	Invasive2	LSD
pH	5.83 ± 0.13	5.31 ± 0.53	6.43 ± 0.21	6.89 ± 0.15	0.21
TOC (g kg⁻¹)	3.30 ± 0.46	3.25 ± 0.46	7.46 ± 1.06	9.21 ± 0.74	1.12
N (g kg⁻¹)	0.10 ± 0.04	0.10 ± 0.04	0.60 ± 0.07	0.70 ± 0.05	0.11
P (mg kg⁻¹)	2.83 ± 1.06	2.53 ± 1.04	5.50 ± 1.42	7.76 ± 1.11	0.98

AMF sporulation, AMF diversity were higher in the Native root zone compared with the results observed in the root zones from different stages of invasion (Invasive1, Invasive2, Exotic) (Tab. 1). The invader altered the composition of AMF communities promoting species from Order Glomerales (e.g. *Rhizoglomus intraradices*) at the expense of AMF species from Order Diversisporales (e.g. genus *Dentiscutata* and *Scutellospora*) (Tab. 2). Our results about AMF diversity and dominance agree with the work done by Oehl *et al.* (2010) that studied the dynamics of AMF sporulation and found that the AMF community was more diverse in natural fields and with other studies on invasive exotic plants (Hawkes *et al.* 2006). The presence of *C. madagascariensis* changed AMF composition in roots of *C. prunifera* along the invasion gradient, and when coexisting with the native species, the invader was able to kill the palm tree during its growth, changing the abundance of spores, and AMF diversity (Tab. 1), and the frequency of occurrence of different AMF species, like *Acaulospora, Dentiscutata, Entrophospora, Gigaspora,* and *Scutellospora* genus (Tab. 2)

The invasion also altered the values for soil pH, total organic carbon, total nitrogen, and available phosphorous in the root zone (Tab. 3). AMF species from Order Diversisporales (genus *Acaulospora*), and Gigasporales (genus *Dentiscutata, Gigaspora*, and *Scutellospora*) are common in semi-arid sites (Dandan & Zhiwei 2007; Silva *et al.* 2014), and in acid soil with low phosphorous availability (Ramos *et al.* 2008). However, disturbances and changes in soil properties, such as soil pH, total organic carbon and available phosphorous can be favorable for Glomerales species (Oehl *et al.* 2010; Jansa *et al.* 2014; Carneiro *et al.* 2015). Therefore, changes in soil properties induced by the invasive plant may contribute to constrain the occurrence of some AMF species, reduce AMF sporulation, and decrease AMF diversity and AMF dominance (Tab. 1). Silva *et al.* (2014) in a study performed in an environmental gradient in the Brazilian semi-arid showed a strong correlation of AMF community composition with soil attributes and soil properties are influenced by plant community on a local scale, affecting the community structure even in areas that are geographically close. In our study, the presence of high density of *C. prunifera* dead roots might have stimulate the sporulation specific AMF species and might accelerate chemicals processes in the rhizosphere soil that changed

the soil properties, like soil pH, total organic carbon, total nitrogen and available phosphorous (Tab. 3).

Studies also showed that plant neighbors were important in structuring AMF communities (Hausmann & Hawkes 2009), the presence of invasive plants changed AMF composition in roots of their native neighbors (Hawkes *et al.* 2006), and soil properties also can have influence on the AMF community composition (Oehl *et al.* 2010; Jansa *et al.* 2014; Silva *et al.* 2014).

In our bioassay, all inoculation treatments were capable of root colonization of *C. madagascariensis* plants. Plant dry biomass and root colonization (Figs. 1-2), indicated that the host-plant responded differently to the six AMF treatments. The results from these two variables also indicated a significant responsiveness (around 52 %) of *C. madagascariensis* to *Rhizoglomus intraradices* inoculation. In fact, we found a positive effect of *Rhizoglomus intraradices* in the invasive plant, which might promote the success of the invader (Figs. 4-5).

Prior studies have shown that invasive plants can alter AMF-plants mutualisms (Stinson *et al.* 2006; Callaway *et al.* 2008; Seifert *et al.* 2009 Vogelsang & Bever 2009). In our study, we found that the inoculation with AMF communities from invaded soils and with *R. intraradices* resulted in a higher plant dry biomass (Control < Native < Exotic = Invasive1 < Invasive2 < Rhi), root colonization (Native < Exotic = Invasive1 = Rhi = Invasive2) (Fig. 3), and phosphorous uptake (Native < Control < Exotic = Invasive1 < Invasive2 < Rhi) (Fig. 4) than inoculation with native AMF community from *C. prunifera* rhizosphere.

Our work increases the understanding of the ecological mechanisms underlying how exotic species can alter the AMF community composition in field conditions; and how modified AMF communities can affect the growth of the invader. Invaders can encounter certain AMF species that can facilitate their establishment in new environments (Reinhart & Callaway 2006; CMR Pereira *et al.* 2014) or the invader can alter AMF composition so as to reduce native species' competitiveness (Vogelsang & Bever 2009; Stinson *et al.* 2006). Our results indicate that, under field conditions, *C. madagascariensis* can change important AMF species either due to a direct preference for some AMF species or indirectly by changes in soil properties. This shift in the AMF community generates positive feedback with the invasive plant as demonstrated in the bioassay experiment. Based on these results, we suggest that the strategy used by *C. madagascariensis* to occupy new environments is to increase the abundance of *Rhizoglomus intraradices* which in turn has a highly positive effect on the invader´s growth.

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