**A NURSE PLANT BENEFITS FROM FACILITATIVE INTERACTIONS
THROUGH MYCORRHIZAE¹**

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ABSTRACT

- Plant facilitation promotes coexistence by maintaining differences in the regeneration niche because some nurse species recruit under arid conditions, whereas facilitated species recruit under more mesic conditions. In one Mexican community, 95% of species recruit through facilitation, being *Mimosa luisana* a keystone nurse for many of them. *M. luisana* individuals manifest greater fitness when growing in association with their facilitated plants than when growing in isolation. This observation suggests that nurses also benefit from their facilitated plants, a benefit thought to be mediated by mycorrhizal fungi.
- Under field conditions, we experimentally tested whether mycorrhizal fungi mediate the increased fitness that *M. luisana* experiences when growing in association with its facilitated plants. We applied fungicide to the soil for nurse plants growing alone and growing in association with their facilitated plants in order to reduce the mycorrhizal colonization of roots. We then assessed the quantity and quality of seed production of *M. luisana* in four treatments (isolated-control, isolated-fungicide, associated-control and associated-fungicide).
- Fungicide application reduced the percent root length colonized by mycorrhizae and reduced fitness of *M. luisana* when growing in association with their facilitated plants but not when growing in isolation. This reduction was reflected in the total number of seeds, number of seeds per pod, seed mass and seed viability.
- These results suggest that nurses are benefitting from the presence of their facilitated plants through links established by mycorrhizae, indicating that both

plants and belowground, mutualistic communities are all part of one system,
coexisting by means of intrinsically linked interactions.

INTRODUCTION

The recognition that aboveground and belowground communities form major components of ecosystems interacting in a permanent feedback has been a major step towards a comprehensive understanding of species coexistence and vegetation dynamics (Wardle *et al.* 2004; Sanon *et al.* 2009; Van der Putten 2009; Bardgett & Wardle 2010; Fukami & Nakajima 2013; Van der Putten *et al.* 2013). For example, plant diversity can be explained in terms of a process of soil community feedback, through which the density and composition of microbes are altered in response to the composition of plant community and subsequently, the change in microbial composition modifies the performance of plants (Bever *et al.* 2010).

Plant species coexistence in stressful environments is mediated by facilitative interactions among plants that are tightly linked to the diversity of belowground microbial communities (Montesinos-Navarro *et al.* 2012a; Montesinos-Navarro *et al.* 2015; Rodríguez-Echeverría *et al.* 2015). Facilitative interactions result in strong spatial associations between a variable number of species and a nurse species. A high proportion of these early associations are maintained through time until facilitated plants reach the adult stage forming discrete vegetation patches surrounded by open space (Valiente-Banuet & Verdú 2007). These vegetation patches are ubiquitous in many ecosystems (Hacker & Gaines 1997; Eccles *et al.* 1999; Castillo *et al.* 2010) and constitute the context in which new individuals belonging to different species arrive via seed dispersal. Species coexistence

in vegetation patches will ultimately be determined by the relative performance of the newcomers and the nurse species. In this process, the role of mycorrhizal fungi in interconnecting plants providing pathways for the transference of nutrients has acquired a central interest to understand benefits among the species involved in the plant facilitation process (Van der Heijden & Horton 2009; Van der Putten 2009; Montesinos-Navarro *et al.* 2012b).

Most studies have focused on the mycorrhizal network effects on the facilitated seedlings (Nara & Hogetsu 2004; Dickie *et al.* 2006) but nurses are also plugged to the network and can receive either positive or negative effects. Previous studies show the mutual benefit in facilitative interactions. For example, Pugnaire *et al.* (1996) showed that *Marrubium vulgare* individuals increase its specific leaf area, leaf mass, shoot mass, nitrogen content, and produce more flowers when grow associated to the shrub *Retama sphaerocarpa*. Similarly, the biomass of 1-yr-old cladodes, total biomass, and total nitrogen content of 3-yr-old branches of *R. sphaerocarpa* shrubs were higher when *M. vulgare* individuals were growing beneath them.

Similarly, the nurse *Mimosa luisana* produces more and better seeds when growing associated to its facilitated species in phylogenetically diverse neighborhoods (Sortibrán *et al.* 2014) while the facilitated species receive N from the nurse (Montesinos-Navarro *et al.* 2016, 2017). Positive effects of facilitated plants on nurses would explain the maintenance of facilitative interactions until adult stages in vegetation patches, and such effects could be mediated by mycorrhizal fungi (Verdú *et al.* 2010). Mycorrhizal fungi can transport water, nutrients and chemical defenses from facilitated plants directly to their nurses through common mycorrhizal networks (Newman 1988; Selosse *et al.* 2006; Simard *et al.* 2012; Gorzelak *et al.* 2015) or indirectly through hydraulic lift or hyphal exudates (Querejeta *et*

94 *al.* 2003; Finlay 2008; Zhang *et al.* 2016). Direct transfer of nutrients and water between
95 plant roots through mycorrhizal networks has been extensively documented (Giovannetti *et*
96 *al.* 2004; Egerton-Warburton *et al.* 2007; Mikkelsen *et al.* 2008; Simard *et al.* 2012;
97 Montesinos-Navarro *et al.* 2016) but its ecological role on the outcome of plant-plant
98 interactions and community dynamics is still debated (van der Heijden & Horton 2009;
99 Bever *et al.* 2010). Indirect transfer may also occur as mycorrhizal fungi can move water
100 from roots to soil, enhancing the magnitude of hydraulic lift and making water available for
101 other plants (Querejeta *et al.* 2003; Prieto *et al.* 2012). Similarly, mycorrhizal exudates
102 from facilitated plants may alter bacterial communities and accelerate mineralization rates,
103 increasing the availability of nutrients in soil (Zhang *et al.* 2016). All of these mechanisms
104 may explain how nurses can benefit from their facilitated plants through mycorrhizal fungi.
105 However, there is contradictory evidence about the costs of plant facilitation for nurse
106 species along the ontogeny of the interacting plants. In some cases, plant facilitation shifts
107 to competition and nurse species fitness is reduced (Schöb *et al.* 2014a, 2014b, 2014c),
108 which contrasts with other situations where facilitation shifts to mutualism and nurse
109 fitness increases (Sortibrán *et al.* 2014). Aboveground factors (e.g. light, pollinators,
110 herbivores) have been shown to drive the positive and negative feedback effects of
111 beneficiary species on their nurses (Warlde *et al.* 2004; Wolfe *et al.* 2005; Van der Putten
112 2009), but belowground drivers, such as plant-soil positive and negative feedbacks may
113 also be at work (Bever *et al.* 2010). Indeed, the response of plants to mycorrhizal fungi
114 ranges from mutualism to parasitism (Hoeksema *et al.* 2010) and therefore both positive
115 and negative effects of mycorrhizae may be context dependent, depending on plant and
116 fungal species identity as well as on soil nutrient availability (Van der Heijden & Horton
117 2009).

Here, we explore the role of arbuscular mycorrhizal fungi (AMF) involving facilitative interactions between a keystone nurse, *Mimosa luisana*, and its facilitated plant species in a Mexican desert community governed by facilitation (Valiente-Banuet & Verdú 2007; Verdú *et al.* 2010; Verdú & Valiente-Banuet 2011). In this community, *M. luisana* is the only species able to recruit on the open ground and therefore it acts as the primary nurse for most of the species (Verdú & Valiente-Banuet 2008). Individuals of *M. luisana* growing isolated can also be found to be in smaller proportion than the individuals growing associated. Interestingly, *M. luisana* individuals coexisting with their facilitated species have been shown to have greater fitness than isolated individuals (Sortibrán *et al.* 2014), although it remains unclear whether these benefits are mediated by AMF.

Mimosa luisana is the generalist species with the greatest number of partners in the plant-arbuscular mycorrhizal fungal community network (Montesinos-Navarro *et al.* 2012b). Likewise, there is evidence that AMF promote nitrogen transfer from *M. luisana* to its facilitated species (Montesinos-Navarro *et al.* 2016), thus making possible for facilitated species to reciprocate their benefits with the nurse through mycorrhizal fungi along the ontogeny of the interacting plants. We experimentally applied a fungicide to reduce AMF abundance in *M. luisana* nurses growing both in isolation and associated with their facilitated plants and evaluated the effect on its different fitness components (reproductive success). Based on previously explained observations, we hypothesized that a reduction in mycorrhizal fungi abundance will produce a decrease in fitness among nurses when growing in association with their facilitated plants.

MATERIALS AND METHODS

Study area and species—This study was conducted in the semiarid Valley of Zapotitlán (18° 20'N, 97° 28'W), a local basin in the Tehuacán-Cuicatlán Valley of Puebla state, Mexico. This region owes its aridity to the rain shadow produced by the Eastern Sierra Madre (Valiente-Banuet *et al.* 2000). It has an annual average rainfall of 380 mm, most of which falls during the summer months, and an annual mean temperature of 21° C with rare frosts (García 1988). The study site is a xeric shrubland located within the Botanical Garden “Helia Bravo Hollis”, a natural unmodified protected area approximately 30 km south of Tehuacán city. This vegetation is dominated by the columnar cactus *Neobuxbaumia tetetzo* and shrub species such as *Mimosa luisana*, *Mascagnia parviflora*, *Ipomoea arborescens*, *Aeschynomene compacta*, *Caesalpinia melanadenia*, *Calliandra eriophylla*, *Zapoteca formosa*, *Senna wislizenii*, *Agave marmorata*, *A. macroacantha*, *Jatropha neopauciflora*, among other taxa (Valiente-Banuet *et al.* 2000). All of these species, with the exception of *M. luisana*, recruit through facilitation. *M. luisana* is able to facilitate 48 out of 56 species recorded for this community (Verdú & Valiente-Banuet 2008), forming discrete vegetation multispecific patches of up to 12 species and 42 individuals. The area of the vegetation patches ranges from 1 to 10 m² and all facilitated individuals are located beneath the vertical projection of the *M. luisana* canopy. Certain isolated *M. luisana* individuals can also be found separated from other vegetation patches by 1.5-2 meters (see Supporting information S1). Previous work at the study site has characterized the interactions between plants and AMF as a bipartite network characterized by a non-random, nested topology, in which *M. luisana* acts as a hub connected to many AMF nodes (Montesinos-Navarro *et al.* 2012a). Moreover, up to 165 ± 16 AMF spores have been reported under the canopy of *M. luisana* (per 100 g dry weight of soil) from the genera *Glomus* and *Acaulospora* (Camargo-Ricalde & Dhillon 2003).

Mimosa luisana is a deciduous spiny shrub reaching heights of up to 2.2 m. Reproduction initiates when plants reach 0.80 m tall. Pink flowers which grow in spike inflorescences are bisexual and are produced during June, just before the beginning of the rainy season (May-August). Fructification occurs from August to October. The fruit consists of one carpel pod, with each pod containing 2 to 8 seeds; pods are grouped into bunches of 2 to 10 (Grether *et al.* 2006).

Field experiment—Sampled vegetation patches were distributed within an area of ca. 750 m² (15 × 50 m) on an east facing slope with an angle of inclination of between 5 to 10°. Nitrogen and phosphorus concentrations in bare soils, as well as in soil newly colonized by *M. luisana* individuals, are low, ranging from 1.4 to 1.6 g Kg⁻¹ and 11.95 to 12.55 mg Kg⁻¹ respectively (Sortibrán *et al.* 2014). Soil fertility in plots with bare soils and plots where seedlings of *M. luisana* have become established do not differ statistically, indicating that this species is able to recruit in the unfertile conditions of open ground (Sortibrán *et al.* 2014).

To test whether fitness benefits obtained by the nurse *M. luisana* from its facilitated plants are mediated by mycorrhizae, we applied a soil fungicide treatment in randomly selected vegetation patches where reproductive individuals of *M. luisana* (> 80 cm height) lived isolated or associated with neighbors of different species. The experimental design followed a 2 × 2 factorial design, crossing fungicide treatment (fungicide vs. control) with growing condition (alone vs. associated). The sample sizes of each group were: i) control-alone ($n=9$ patches), ii) fungicide-alone ($n=9$), iii) control-associated ($n=14$) and iv) fungicide-associated ($n=20$). This unbalanced design corresponded to the natural

abundance of isolated and associated individuals. The fungicide used was Rovral 50% (Iprodione), Bayer México, a contact fungicide with systemic effects, very efficient for eliminating mycorrhizae without affecting soil, insects, bacteria or plant growth (Gange *et al.* 1990; Ganade & Brown 1997; Hernández-Dorrego & Mestre-Parés 2010). The fungicide was applied at the rate of 2.0 g L⁻¹ water (Ganade & Brown 1997) at intervals of three weeks, prior to the rainy season (February-July) and six times over two years (2011 and 2012) to avert fungicide runoff with rain. In the control treatment, the same amount of water was added as in the fungicide treatments. Fungicide and water were administered in each patch through 6 L canisters inverted into the soil in order to slowly dispense the liquid through a small orifice. To ensure that the fungicide reached all roots in the patch, one canister was set up for each m², across the entire soil surface below the canopy of *M. luisana*. Root samples for AMF colonization measurements and *M. luisana* reproduction records were conducted during September 2012, two months after the last fungicide application.

Fungicide efficacy on AMF colonization—The efficacy of fungicide for reducing AMF abundance on *M. luisana* roots was evaluated by obtaining root samples from all *M. luisana* individuals by excavating three roots as far as the fine roots and cutting twenty fragments 2 cm in length per plant. We estimated the reduction in the percentage of root colonization of AMF following the Phillips & Hayman (1970) method, but in which root samples were stored in 50% ethanol in September 2012. In the laboratory, roots were cleared in a 10% aqueous solution of KOH (w/v) for 10 min under pressure at 120° C, washed with 10% H₂O₂ and acidified with 1% HCl (v-v). Subsequently, roots were stained with 0.05% Trypan blue (w-v) in lactoglycerol. The percentage of root length colonized by AMF was estimated

according to the method outlined by Phillips & Hayman (1970) and Giovannetti & Mosse (1980) by placing the 20 root samples from each plant on slides and examined at 400× magnification, using an optical light microscope B201, Olympus, USA. A total of 100 fields were examined per individual. The percentages of hyphae and vesicles were scored separately by counting the fields in which those structures were present.

Fungicide effect on reproductive success of *Mimosa luisana*—We estimated the effect of fungicide treatment on associated and isolated individuals of *Mimosa luisana*, considering four different aspects of reproductive success: seed production, seed set, seed mass, and seed viability. Seed production was assessed by counting the total number of seeds produced by each individual in the different treatments. Seed set was estimated by counting the seeds per pod in 30 pods from 5 individuals, randomly selected from each combination of treatments. Thirty seeds from each of those five individuals were weighed in order to estimate average seed mass and germinated to assess seed viability. Seeds were scarified with sandpaper prior to germination tests performed in Petri dishes with wet filter paper, at 25 °C in 12 h light/dark. As the number of fruits may depend on the size of the plant, we estimated *M. luisana* shrub size in order to consider this as a covariate in subsequent statistical models. Plant size was estimated by assuming that *M. luisana* is an inverted cone with an elliptical base and calculating volume after measuring the height and two perpendicular diameters of the canopy cover.

Statistical analysis—We compared the effects of treatment (control vs. fungicide) and the growing condition of *M. luisana* (alone vs. associated) on AMF root colonization and on

different components of reproductive output of *M. luisana*, applying Generalized Linear Models (GLM) with different error structures, depending on the nature of the dependent variable. For variables recording presence vs. absence (i.e., hyphae or vesicles in roots observed in 100 fields under the light microscope) or success vs failure (i.e., seed germination), we used a binomial distribution of errors. To account for overdispersion of the model, we used the quasi-binomial family in the GLM. For calculations such as total number of seeds or number of seeds per pod, produced by *M. luisana* individuals, we applied a quasi-Poisson error distribution. Finally, for continuous variables, such as seed mass, we used Gaussian distribution of errors. The size of each plant was included in all the models as a co-variable, but later removed because it did not prove significant in any case. Both normality of residuals and absence of non-linear relationships between fitted values and residuals were verified. All statistical analyses were performed with R software version 3.2.1 (R Development Core Team 2015).

RESULTS

The fungicide significantly reduced AMF hyphal colonization in the roots of *M. luisana* individuals growing in isolation (68% reduction) or associated with other species (78% reduction) (Table 1; Fig 1A). Similarly, vesicle colonization was significantly reduced after fungicide application of 66% and 88% in isolated and associated *M. luisana* individuals, respectively (Table 1; Fig 1B). Following fungicide treatment, similar low levels of AMF were present in the roots of both associated and isolated *M. luisana* individuals.

The effect of fungicide on the reproductive output of *M. luisana* was dependent on growing conditions (isolated and associated) in terms of all four aspects (total number of seeds, seeds per pod, seed mass, and seed viability) as indicated by significant statistical

interactions in Table 1. The fungicide reduced the number of seeds in *M. luisana* individuals growing associated with their facilitated plants by 2.4 times but had no effect on those growing in isolation (Fig. 1C). Similarly, the fungicide significantly reduced the number of seeds per pod in the associated but not in the isolated *M. luisana* individuals (Fig. 1D). Following the same trend, seed mass of *M. luisana* individuals, growing in association was reduced 1.6 times by fungicide, but no effect was detected in individuals growing in isolation (Fig. 1E). Finally, fungicide reduced the seed viability of associated *M. luisana* individuals by 2.8 times, whereas it did not affect the seed viability of isolated individuals (Fig. 1F).

DISCUSSION

Our results mostly show that *M. luisana* nurses obtain fitness benefits partly from their facilitated adult plants through mycorrhizae, illustrating a mechanism by which a facilitative interaction may also be beneficial for the nurse and how plant-plant and belowground positive interactions underlie species coexistence.

Fungicide application significantly reduced seed quantity and quality of *M. luisana* when growing associated with their facilitated plants but not when growing in isolation. When fungicide was applied, AMF colonization of *M. luisana* roots was reduced, possibly diminishing the benefits received from their facilitated plants and, ultimately, reducing the number of seeds per individual, number of seeds per pod, seed mass and seed viability. As the quantity and quality of seeds are indicators of how the plant allocates resources according to nutrient availability in soil (Sinclair & Vadez 2002; Marschner 2012; Hushof *et al.* 2012; DiManno & Ostertag 2016), our results strongly suggest that nurses living with their facilitated plants have greater access to resources *via* mycorrhiza. Similarly, fungicide

could be negatively affecting the facilitated plants because the reduction of their extra-radical mycelia decreases the volume of soil to explore and disrupt the connections with *M. luisana* through which nitrogen is received (Montesinos-Navarro et al (2016). These negative effects of fungicide on facilitated plants would also impact indirectly on the nurse by disrupting the benefits of mycorrhizal fungi. Fungicide may have also produced other effects, such as a reduction in pathogenic fungi and increased nutrient availability, due to decomposition of dead microorganisms as a source of organic matter (Allison *et al.* 2007; Berns *et al.* 2008; Goberna *et al.* 2011). However, if these effects were important, the fungicide treatment should have produced a benefit for *M. luisana* in terms of increased seed production and/or seed quality. In contrast, our results indicate that fungicide had negative effects on the associated nurses.

All our results indicate that nurses benefit from their facilitated plants by means of mycorrhizal fungi. The nurse *M. luisana* is a key species in the study system because it facilitates the establishment of 86% of species in the community (Verdú & Valiente-Banuet 2008) and harbors the highest diversity of AMF (Montesinos-Navarro *et al.* 2012a). *M. luisana*, by facilitating other species, may increase the abundance and diversity of fungi in the rhizosphere. Supporting this idea, we have found that the mean AMF abundance in the nurse (measured as % of root length colonized by AMF hyphae) increases with the number of facilitated species in the patch ($r=0.72$; $p<0.05$). Such increment may allow *M. luisana* to benefit directly or indirectly from the complementary functions provided by different fungi (van der Heijden & Horton 2009; Gorzelak *et al.* 2015). Experimental evidence indicates that plants colonized by multiple AMF species obtain more phosphorus and grow more than those colonized by only one species (van der Heijden *et al.* 1998; Johnson *et al.* 2004; Jansa et al. 2008). A possible mechanism for which enriched rhizospheres benefit plants is

the transfer of nutrients between plants (Garg & Chandel 2010; Simard *et al.*, 2012). Soil-derived nutrients are not only essential for the development of the AMF but are also transferred in part to the host plant itself (Smith & Read 1998; Leake *et al.* 2004; Selosse *et al.* 2006; Bucher 2007; Simard *et al.* 2012). Mycorrhizal fungi may also redistribute water in the soil, increasing the rate of diffusion and the absorption of nutrients (Egerton-Warburton *et al.* 2008).

Two lines of evidence support the existence of mutual benefits between *M. luisana* and its facilitated plants through an interchange of nutrients and water in our study system. First, by using ^{15}N stable isotope, Montesinos-Navarro *et al.* (2016) showed that soil fungi promote nitrogen transfer from *M. luisana* to its facilitated species, while an experiment using deuterium-labelled water showed that facilitated plants may transfer water to *Mimosa luisana* (Alicia Montesinos-Navarro *et al.* unpublished). Similarly, it is possible that other nutrients are transferred between neighboring plants via mycorrhizal fungi (Selosse *et al.* 2006; Van de Heijden & Horton 2009; Mikkelsen *et al.* 2008; Garg & Chandel 2010; Simard *et al.* 2012). Most of these works suggest that nutrient transfer between plants occurs through common mycorrhizal networks that physically integrate them. However, hyphal linkages in field experiments like ours are difficult to demonstrate (Perry 1995), and other mechanisms may also explain how mycorrhizal fungi mediate the fitness benefits of the nurses when living in close association with their facilitated plants. For example, our results might be explained by increased production of extraradical mycelia in *M. luisana* individuals associated with their facilitated plants, as a result of multiple nutrients provided to fungi by facilitated plant species. More extensive extraradical mycelia may increase water and nutrient absorption along hyphae, ultimately influencing nurse fitness. However, when *M. luisana* grows in isolation, sources of nutrients for fungi are more limited and

extra-radical hyphal networks may not be as extensive as most of soil microorganisms are carbon starved (Berendsen et al. 2012). Thus, the development of the extra-radical hyphal network in isolated *M. luisana* individuals may be carbon limited because a high maintenance cost, which may be up to 40% of photosynthates secreted into the rhizosphere (Bais et al. 2006). However, once facilitated species grow associated and connected to *M. luisana* through the hyphal network, a supplementary source of nutrients may be available for fungi allowing the formation of a more extensive mycelia. This difference in the size of extra-radical mycelia between nurses growing in association or isolation may explain why fungicide reduced the fitness of the former, but not that of the latter. Similarly, an extensive mycorrhizal network would enable the nurse to have greater access to the water and nutrients that their facilitated species may deliver through hydraulic lift and root exudates, processes in which mycorrhizal fungi can also participate (Prieto et al. 2012). Unfortunately, using our data, it is impossible to separate the relative contributions made by these non-exclusive mechanisms. However, an unequivocal pattern of nurse benefits, apparently mediated by mycorrhizal fungi, is revealed that deserves further research. Two potentially confounding factors to explain the increment of the quantity and quality of seed production for *M. luisana* individuals associated to its facilitated species are that nurse plants with associated species might be older or inhabit preferable microsites, where mycorrhizae are more abundant. However, our analyses show that plant size, as a proxy of age, does not correlate significantly with quantity and quality of seed production in any case. Furthermore, microclimatic variation in the study site was tested previously by Sortibrán et al. (2014) by analyzing soils samples taken under *M. luisana* saplings (ca. 10 cm height) and in open areas, showing that improved soil condition under *M. luisana* adults is not a cause of initial recruitment. Consequently, we may discard also this confounding

effect by showing that microhabitat amelioration is a direct effect, and not the cause of nurse establishment.

In short, contrary to traditional thinking, which suggests that competition is the main mechanism assembling ecological communities, our results show that within the context of plant facilitation, plants and belowground mutualistic communities are all part of one system, whose positive interactions ultimately determine coexistence.

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REFERENCES

Allison V.J., Rajaniemi T.K., Goldberg D.E., Zak D.R. (2007) Quantifying direct and indirect effects of fungicide on an old-field plant community: an experimental null-community approach. *Plant Ecology*, **190**, 53–69.

- 379 Bais H.P., Weir T.L., Perry L.G., Gilroy S., Vivanco J.M. (2006) The role of root exudates
380 in rhizosphere interactions with plants and other organisms. *Annual Review of Plant*
381 *Biology*, **57**, 233–66.
- 382 Bardgett, R.D., Wardle D.A. (2010) *Aboveground-belowground linkages: biotic*
383 *interactions, ecosystem processes, and global change*. Oxford University Press,
384 Oxford, UK: 320 pp.
- 385 Berns A.E., Philipp, H., Narres H.D., Burauel P., Vereecken H., Tappe W. (2008) Effect of
386 gamma-sterilization and autoclaving on soil organic matter structure as studied by
387 solid state NMR UV and fluorescence spectroscopy. *European Journal*
388 *of Soil Science* **59**, 540–550.
- 389 Bever J.D., Dickie I.A., Facelli E., Facelli J.M., Klironomos J., Moora M., Rillig
390 M.C., Stock W.D., Tibbett M., Zobel M. (2010) Rooting theories of plant
391 community ecology in microbial interactions. *Trends Ecology and Evolution*, **25**,
392 468–478.
- 393 Berendsen R.L., Pieterse C.M.J., Bakker A.H.M. (2012) The rhizosphere microbiome and
394 plant health. *Trends in Plant Science*, **17**, 478–486.
- 395 Bucher M. (2007) Functional biology of plant phosphate uptake at root and mycorrhiza
396 interfaces. *New Phytologist*, **173**, 11–26.
- 397 Camargo-Ricalde S.L., Dhillon S.S., Jiménez-González C. (2003) Mycorrhizal perennials
398 of the “matorral xerófilo” and the “selva baja caducifolia” communities in the
399 semiarid Tehuacán-Cuicatlán Valley, Mexico. *Mycorrhiza*, **13**, 77–83.
- 400 Castillo L.J.P., Verdú M., Valiente-Banuet A. (2010) Neighborhood phylodiversity affects
401 plant performance. *Ecology*, **91**, 3656–3663.

- 402 Chesson P. (2000) Mechanisms of maintenance of species diversity. *Annual Review of*
403 *Ecology and Systematics*, **31**, 343–366.
- 404 Dickie I.A., Oleksyn J., Reich P.B., Karolewski P., Zytkowski R., Jagodzinski A.M.,
405 Turzanska E. (2006) Soil modification by different tree species influences the extent
406 of seedling ectomycorrhizal infection. *Mycorrhiza*, **16**, 73–79.
- 407 DiManno N., Ostertag R. (2016) Reproductive response to nitrogen and phosphorus
408 fertilization along the Hawaiian archipelago's natural soil fertility gradient.
409 *Oecologia*, **180**, 245–255.
- 410 Eccles N.S., Esler K.J., Cowling R.M. (1999) Spatial pattern analysis in Namaqualand
411 desert plant communities: evidence for general positive interactions. *Plant Ecology*,
412 **142**, 71–85.
- 413 Egerton-Warburton L.M., Querejeta J.I., Allen M.F. (2007) Common mycorrhizal networks
414 provide a potential pathway for the transfer of hydraulically lifted water between
415 plants. *Journal of Experimental Botany*, **58**, 1473–1483.
- 416 Egerton-Warburton L.M., Querejeta J.I., Allen M.F. (2008) Efflux of hydraulically lifted
417 water from mycorrhizal fungal hyphae during imposed drought. *Plant Signalling &*
418 *Behaviour*, **3**, 68–71.
- 419 Finlay R.D. (2008) Ecological aspects of mycorrhizal symbiosis: with special emphasis on
420 the functional diversity of interactions involving the extraradical mycelium. *Journal*
421 *of Experimental Botany*, **59**, 1115–1126.
- 422 Fukami T., Nakajima M. (2013) Complex plant–soil interactions enhance plant species
423 diversity by delaying community convergence. *Journal of Ecology*, **101**, 316–324.

- 424 Ganade G., Brown V.K. (1997) Effects of below-ground insects, mycorrhizal fungi and soil
 425 fertility on the establishment of *Vicia* in grassland communities. *Oecologia*, **109**,
 426 374–381.
- 427 Gange A.C., Brown V.K., Aplin D.M (1990) A test of mycorrhizal benefit in an early
 428 successional plant community. *New Phytologist*, **115**, 85–91.
- 429 García E. (1988) *Modificaciones al sistema de clasificación climática de Köppen. para*
 430 *adaptarlo a las condiciones de la República Mexicana*. Instituto de Geografía,
 431 Universidad Nacional Autónoma de México, México.
- 432 Garg N., Chandel S. (2010) Arbuscular mycorrhizal networks: process and functions.
 433 *Agronomy for Sustainable Development*, **30**, 581–599.
- 434 Giovannetti M., Mosse B. (1980) An evaluation of techniques for measuring vesicular-
 435 arbuscular mycorrhizal infection in roots. *New Phytologist*, **84**, 489–500.
- 436 Giovannetti M., Sbrana C., Avio L., Strani P. (2004) Patterns of below-ground plant
 437 interconnections established by means of arbuscular mycorrhizal networks. *New*
 438 *Phytologist*, **164**, 175–181.
- 439 Goberna M., Podmirseg S.M., Waldhuber S., Knapp B.A., García C., Insam H. (2011)
 440 Pathogenic bacteria and mineral N in soils following the land spreading of biogas
 441 digestates and fresh manure. *Applied Soil Ecology*, **49**, 18–25.
- 442 Gorzelak M.A., Asay A.K., Pickles B.J., Simard S.W. (2015) Inter-plant communication
 443 through mycorrhizal networks mediates complex adaptive behaviour in plant
 444 communities, *AoB Plants*, **7**(1) plv050.
- 445 Grether R., Martínez-Bernal A., Luckow M., Zárate S. (2006) *Flora del valle de Tehuacán-*
 446 *Cuicatlán, MIMOSACEAE Tribu Mimoseae*. Instituto de Biología, UNAM **44**, 1–
 447 108.

- 448 Hacker S.D., Gaines M.D. (1997) Some implications of direct positive interactions for
449 community species richness. *Ecology*, **78**, 1990–2003.
- 450 Hernández-Dorrego A., Mestre-Parés P. (2010) Evaluation of some fungicides on
451 mycorrhizal symbiosis between two *Glomus* species from commercial inocula and
452 *Allium porrum* L. seedlings. *The Journal of Agriculture Research*, **8**, 43–50.
- 453 Hoeksema J.D., Chaudhary V.B., Gehring C.A., Johnson N.C., Karst J., Koide R., Pringle
454 A. (2010) A meta-analysis of context-dependency in plant response to inoculation
455 with mycorrhizal fungi. *Ecology Letters*, **13**, 394–407.
- 456 Hulshof C.M., Stegen J.C., Swenson N.G., Enquist C.A.F., Enquist B.J. (2012) Interannual
457 variability of growth and reproduction in *Bursera simaruba*: the role of allometry
458 and resource variability. *Ecology*, **93**, 180–190.
- 459 Jansa J., Smith F.A., Smith S.E. (2008) Are there benefits of simultaneous root colonization
460 by different arbuscular mycorrhizal fungi? *New Phytologist*, **177**, 779–789.
- 461 Johnson D., Vandenkoornhuyse P.J., Leake J.R., Gilbert L., Booth R.E., Grime, Young
462 J.P.W., Read D.J. (2004) Plant communities affect arbuscular mycorrhizal fungal
463 diversity and community composition in grassland microcosms. *New Phytologist*,
464 **161**, 503–515.
- 465 Leake J.R., Johnson D., Donnelly D., Muckle G., Boddy L., Read D. (2004) Networks of
466 power and influence: the role of mycorrhizal mycelium in controlling plant
467 communities and agroecosystem functioning. *Canadian Journal of Botany*, **82**,
468 1016–1045.

- 469 Marschner P. (2012) *Marschner's Mineral Nutrition of Higher Plants*. 3rd Edition.
470 Elsevier. Oxford, UK: 672 pp.
- 471 Mikkelsen B.L., Rosendahl S., Jakobsen I. (2008) Underground resource allocation
472 between individual networks of mycorrhizal fungi. *New Phytologist*, **180**, 890–898.
- 473 Montesinos-Navarro A, Segarra-Moragues J.G., Valiente-Banuet A., Verdú M. (2012a) The
474 network structure of plant–arbuscular mycorrhizal fungi. *New Phytologist* **194**, 536–
475 547.
- 476 Montesinos-Navarro A., Segarra-Moragues J.G., Valiente-Banuet A., Verdú M. (2012b)
477 Plant facilitation occurs between species differing in their associated arbuscular
478 mycorrhizal fungi. *New Phytologist*, **196**, 835–844.
- 479 Montesinos-Navarro A., Segarra-Moragues J.G., Valiente-Banuet A., Verdú M. (2015)
480 Evidence for phylogenetic correlation of plant-AMF assemblages? *Annals of Botany*,
481 **115**, 171–177.
- 482 Montesinos-Navarro A., Verdú M., Querejeta J.I., Sortibrán L., Valiente-Banuet A. (2016)
483 Soil fungi promote nitrogen transfer among plants involved in long-lasting
484 facilitative interactions. *Perspectives in Plant Ecology, Evolution and Systematics*,
485 **18**, 45–51.
- 486 Montesinos-Navarro A., Verdú M., Querejeta J.I., Valiente-Banuet A. (2017) Nurse plants
487 transfer more nitrogen to distantly related species. *Ecology*, **98**, 1300–1310.
- 488 Nara K., Hogetsu T. (2004) Ectomycorrhizal fungi on established shrubs facilitate
489 subsequent seedling establishment of successional plant species. *Ecology*, **85**, 1700–
490 1707.

- 491 Newman E.I. (1988) Mycorrhizal links between plants: their functioning and ecological
492 significance. *Advances in Ecological Research*, **18**, 243–270.
- 493 Perry D.A. (1995) Self-organizing systems across scales. *Trends in Ecology and Evolution*,
494 **10**, 241–244.
- 495 Phillips J.M., Hayman D.S. (1970) Improved procedures for clearing and staining parasitic
496 and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection.
497 *Transactions of the British Mycological Society*, **55**, 158–161.
- 498 Prieto I., Armas C., Pugnaire F.I. (2012) Water release through plant roots: new insights
499 into its consequences at the plant and ecosystem level. *New Phytologist*, **193**, 830–
500 841.
- 501 Pugnaire F.I., Haase P., Puigdefabregas J. (1996) Facilitation between higher plant species
502 in a semiarid environment. *Ecology*, **77**, 1420–1426.
- 503 Querejeta J.I., Egerton-Warburton L.M., Allen M.F. (2003) Direct nocturnal water transfer
504 from oaks to their mycorrhizal symbionts during severe soil drying. *Oecologia*, **134**,
505 55–64.
- 506 R Development Core Team. (2015) *R: A Language and Environment for Statistical*
507 *Computing*. R Foundation for Statistical Computing, Vienna, Austria. URL
508 <http://www.R-project.org>.
- 509 Rodríguez-Echeverría S., Lozano Y., Bardgett R.D. (2015) Influence of soil microbiota in
510 nurse plant systems. *Functional Ecology*, **30**, 30–40.
- 511 Sanon A., Andrianjaka Z.N., Prin Y., Bally R., Thioulouse J., Comte G., Duponnois R.
512 (2009) Rhizosphere microbiota interferes with plant-plant interactions. *Plant and*
513 *Soil*, **321**, 259–278.

- 514 Schöb, C., Callaway R.M., Anthelme F., Brooker R., Cavieres L., Kikvidze Z., Lortie C.J.,
 515 Michalet R., Pugnaire F.I., Xiao S., Cranston B.H., García M.C., Hupp N.R.,
 516 Llambí L.D., Lingua E., Reid A.M., Zhao L., Butterfield B.J. (2014a) The context-
 517 dependence of beneficiary feedback effects on benefactors in plant facilitation. *New*
 518 *Phytologist*, **204**, 386–396.
- 519 Schöb C., Michalet R., Cavieres L.A., Pugnaire F.I., Brooker R.W., Butterfield B.J., Cook
 520 B.J., Kikvidze, Z., Lortie C.J., Xiao S., Al Hayek P., Anthelme F., Cranston
 521 B.H., García M.C., Le Bagousse-Pinguet Y., Reid A.M., le Roux P.C., Lingua
 522 E., Nyakatya M.J., Touzard B., Zhao L., Callaway RM. (2014b) A global analysis
 523 of bidirectional interactions in alpine plant communities shows facilitators
 524 experiencing strong reciprocal fitness costs. *New Phytologist*, **202**, 95–105.
- 525 Schöb C., Prieto I., Armas C., Pugnaire F.I. (2014c) Consequences of facilitation: one
 526 plant's benefit is another plant's cost. *Journal of Ecology*, **28**, 500–508.
- 527 Selosse M.A., Richard F., He X., Simard S.W. (2006) Mycorrhizal networks: des liaisons
 528 dangereuses? *Trends in Ecology and Evolution*, **21**, 621–628.
- 529 Simard S.W., Beiler K.J., Bingham M.A., Deslippe J.R., Philip L.J., Teste F.P. (2012)
 530 Mycorrhizal networks: Mechanisms, ecology and modelling.
 531 *Fungal Biology Reviews*, **26**, 39–60.
- 532 Sinclair T.R., Vadez V. (2002) Physiological traits for crop yield improvement in low N
 533 and environments. *Plant and Soil*, **245**, 1–15.
- 534 Smith S.E., Read D.J. (2008) *Mycorrhizal Symbiosis*. 3rd edition. Academic Press, Oxford,
 535 UK: 800 pp.

- Sortibrán L., Verdú M., Valiente-Banuet A. (2014) Nurses experience reciprocal fitness benefits from their distantly related facilitated plants. *Perspectives in Plant Ecology, Evolution and Systematics*, **16**, 228–235.
- Valiente-Banuet A., Casas A., Alcántara A., Dávila P., Flores-Hernández N., Villaseñor J.L., Ortega J. (2000) La vegetación del Valle de Tehuacán-Cuicatlán. *Boletín de la Sociedad Botánica de Mexico*, **67**, 25–74.
- Valiente-Banuet A., Verdú M. (2007) Facilitation can increase the phylogenetic diversity of plant communities. *Ecology Letters*, **10**, 1029–1036.
- Van der Heijden M.A.G., Klironomos J.N., Ursic M., Moutoglis P., Streitwolf-Engel R., Boller T., Wiemken A., Sanders I.R. (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, **396**, 69–72.
- Van der Heijden M.A.G., Horton T. (2009) Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. *Journal of Ecology*, **97**, 1139–1150.
- Van der Putten W.H. (2009) A multitrophic perspective on functioning and evolution of facilitation in plant communities. *Journal of Ecology*, **97**, 1131–1138.
- Van der Putten W.H., Bardgett R.D., Bever J.D., Bezemer T.M., Casper B.B., Fukami T., Kardol P., Klironomos J.N., Kulmatiski A., Schweitzer J.A., Suding K.N., Van de Voorde T.F.J., Wardle D.A. (2013) Plant soil feedbacks: the past, the present and future challenges. *Journal of Ecology*, **101**, 265–276.
- Verdú M., Valiente-Banuet A. (2008) The nested assembly of plant facilitation networks prevents species extinctions. *American Naturalist*, **172**, 75–76.

- 559 Verdú M., Jordano P., Valiente-Banuet A. (2010) The phylogenetic structure of plant
560 facilitation networks changes with competition. *Journal of Ecology*, **98**, 1454–1461.
- 561 Verdú M. Valiente-Banuet A. (2011) The relative contribution of abundance and phylogeny
562 to the structure of plant facilitation networks. *Oikos*, **120**, 1351–1356.
- 563 Wardle D.A., Bardgett R.D., Klironomos J.N., Setälä H., Van der Putten W.H., Wall D.H.
564 (2004) Ecological linkages between aboveground and belowground biota. *Science*,
565 **304**, 1629–1633.
- 566 Wolfe B.E., Husband B.C., Klironomos J.N. (2005) Effects of a belowground mutualism
567 on an aboveground mutualism. *Ecology Letters*, **8**, 218–223.
- 568 Yang H., Yu Z., Zhang Q., Tang J., Chen X. (2012) Plant neighbor effects mediated by
569 rhizosphere factors along a simulated aridity gradient. *Plant and Soil*, **369**, 165–176.
- 570 Zhang L., Xu M., Liu Y., Zhang F., Hodge A., Feng G. (2016) Carbon and phosphorus
571 exchange may enable cooperation between an arbuscular mycorrhizal fungus and a
572 phosphate-solubilizing bacterium. *New Phytologist*, **210**, 1022–1032.
- 573

TABLE 1. Effects of fungicide treatment (control vs fungicide) on AMF root colonization and reproductive output of *M. luisana* individuals growing under different conditions (isolated vs. associated). Each column shows the estimates and SE of the corresponding Generalized Linear Model.

	% Root colonization		Reproductive output			
	Hyphae	Vesicle	Seed number	Seeds/pod	Seed mass	Seed viability
Condition	0.26±0.26	0.41 ±	2.23 ±	0.23 ±	0.47 ±	1.82 ±
		0.28	0.86*	0.05*	0.004*	0.56*
Fungicide	-2.12 ±	-1.31 ±	0.90 ±	0.06 ±	-0.04 ±	-0.03 ±
	0.29*	0.41*	0.99	0.05	0.05	0.65
Condition x Fungicide	-0.73	-1.25 ±	-1.78 ±	-0.02 ±	-0.47 ±	-1.65 ±
	±0.38 ^{ms}	0.56*	1.00 ^{ms}	0.04*	0.06*	0.77*

*p<0.05; ^{ms} 0.05<p<0.10

Figure Legends

FIGURE 1 Fungicide effects on percent root length colonized by A) hyphae; and B) vesicles; C) total seed production; D) seeds per pod; E) seed mass and F) seed germination percentage of *Mimosa luisana* individuals growing alone or associated to their facilitated plants in control and fungicide treatments. Values are means $\pm$ SE. Asterisks indicate significant differences for the effect of fungicide within each treatment (post hoc analysis) at $p < 0.05$.

Supporting Information

FIGURE S1 Study site showing three vegetation patches in which *M. luisana* grows associated multispecifically with the facilitated plants *Neobuxbaumia tetezo*, *Opuntia pilifera*, *Thompsonella minutiflora*, *Mammillaria colina* *Coryphantha pallida* and *Siphonoglossa ramosa* (white circle); growing associated to *T. minutiflora* (yellow circle); and growing in isolation (red circle). The distance between patches ranges from 1.5 to 2.5 m. Photograph by Lugui Sortibrán.

Fig 1.

FIG 1. Fungicide effects on percent root length colonized by A) hyphae; and B) vesicles; C) total seed production; D) seeds per pod; E) seed mass and F) seed germination percentage of *Mimosa luisana* individuals growing alone or associated to their facilitated plants in control and fungicide treatments. Values are means $\pm$ SE. Asterisks indicate significant differences for the effect of fungicide within each treatment (post hoc analysis) at $p < 0.05$.

254x190mm (300 x 300 DPI)

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FIGURE S1