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Soil fungi promote nitrogen transfer among plants involved in long-lasting facilitative interactions

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Abstract

Plant facilitative interactions may persist in the long term when there are benefits for the interacting adult plants. Whereas persistent benefits for adult nurse plants have been demonstrated, the long-term benefits derived by adult facilitated plants have been largely unexplored. We hypothesize that common mycorrhizal networks (CMNs) can provide a pathway through which nurse species can benefit adult facilitated plants persistently. We specifically test whether nitrogen can be transferred from nurse plants to their adult facilitated plants, and evaluate to which extent CMNs mediate the transfer. We selected 32 adult individuals of 6 facilitated plant species growing in 15 vegetation patches in a Mexican desert. We treated some vegetation patches with fungicide and left others as controls. Then, we labeled the nurse plants with 15N-enriched urea and

quantified the amount of 15N transferred to their adult facilitated plants. We expected a greater 15N transfer to facilitated individuals growing in vegetation patches with intact CMNs than in those treated with fungicide. Facilitated plants growing in patches with intact CMNs showed on average a greater increment in their foliar 815N (i.e. difference between post-labeling–pre- labeling) than those in patches treated with fungicide. Our results provide evidence that CMNs enhance nitrogen transfer among adult plants, thus providing a potential mechanism contributing to the long-term persistence of plant facilitative associations.

Introduction

The ecological mechanisms underlying plant facilitative inter- actions are well known, but a deep understanding of their evolutionary implications is still lacking (Brooker et al., 2008, Valiente-Banuet and Verdú, 2013). In order to explore the effects of facilitative interactions in the fitness of the interacting plant species, a first step is to assess whether the benefits resulting from the interaction are maintained along the ontogeny of the interacting plants. In facilitative interactions, at least one of the associated species gets a benefit (facilitated species) without result- ing in any damage for the other (nurse species) or even providing a benefit to it (Callaway, 2007; Sortibrán et al., 2014). Benefits for adult nurse plant have been reported in arid environments, with nurse plants producing more flowers and fruits when grow- ing associated with their facilitated plants than when growing alone (Sortibrán et al., 2014, unpublished results). However, in alpine systems, the nurse plant benefits can shift to costs depend- ing on the reproductive trait measured (Schöb et al., 2014). In addition, while adult nurse plants facilitate the establishment of seedlings of facilitated species, asymmetric competition later on in the ontogeny of the facilitated plants can result in mortality of the adult facilitated plants (Valiente-Banuet and Verdú, 2008; Armas and Pugnaire, 2009; Rolo et al., 2013). However, other facilitative interactions persist over time resulting in adult nurse and facil- itated plants associations (Valiente-Banuet and Verdú, 2008). In persistent interactions which can reach 57% of all the facilitative interactions (Valiente-Banuet and Verdú, 2008), the adult facili- tated plants may still receive some

benefits from being associated with their nurse plant. The mechanisms by which nurse plants can promote facilitated seedlings establishment have received consid- erable attention (Nara and Hogetsu, 2004; Nara, 2006; Richard et al., 2009; Teste et al., 2009; Van der Heijden and Horton, 2009; Booth and Hoeksema, 2010; Bingham and Simard, 2011, 2012; Molina- Montenegro et al., 2015). However, the mechanisms enhancing the benefits for adult facilitated plants have been largely unexplored.

Mycorrhizal fungi may play an important role in the persistence of plant facilitative interactions, considering their influence in the outcome of plant–plant interactions in a wide variety of ecosys- tems, including alpine, Mediterranean, marshland, dune shore, forest, prairies and deserts (Hartnett et al., 1993; Nara and Hogetsu, 2004; Nara, 2006; Richard et al., 2009; Booth and Hoeksema, 2010; Grau et al., 2010; Casanova-Katny et al., 2011; Montesinos-Navarro et al., 2012a; Zhang et al., 2014; Molina-Montenegro et al., 2015). The mycelia of mycorrhizal fungi can colonize the roots of neigh- bor plants, establishing common mycorrhizal networks (CMN) that allow intra and interspecific transference of resources between plants (Newman, 1988; Simard and Durall, 2004; Selosse et al., 2006). Plants connected through CMN can exchange signals pro- moting genes involved in defense against pathogen infection (Song et al., 2010), induce the production of volatiles to protect neighbor plants from herbivores (Babikova et al., 2013), or transfer alle- lochemicals which expands the action range of these regulators of plant competition (Barto et al., 2011). Resource translocation though CMN promotes seedling growth and survival through water and nitrogen transfer from adult donors (Teste et al., 2009; Booth and Hoeksema, 2010; Bingham and Simard, 2011), which in the long term can give rise to emergent patterns at the plant community level. Mycorrhizal fungi can affect plant communi- ties by reducing interspecific competition among co-existing plant species when the diversity of mycorrhizal fungi increases (Wagg et al., 2011). Furthermore, the assembly of plant and mycorrhizal communities seems to be closely interrelated as suggested by a cor- respondence between the phylogenetic structures of mycorrhizal and plant communities in vegetation patches (Montesinos-Navarro et al., 2015). Both mycorrhizal and plant assemblages can in turn influence each other a follows. On the one hand, mycorrhizal species richness can promote plant diversity and productivity (Van der Heijden et al., 1998; Vogelsang et al.,

2006; Maherali and Klironomos, 2007). On the other hand, plant facilitative interactions can indirectly influence mycorrhizal assemblages, as nurses tend to associate with facilitated species that increase the mycor- rhizal richness in the shared rhizosphere (Montesinos-Navarro et al., 2012a). Therefore, the outcome of facilitative interactions can be mediated by the mycorrhizal fungi shared in the rhizo- sphere, and the mycorrhizal community can be in turn shaped by the plant species involved in the facilitative interactions. In this sense, the nurse plant performance is enhanced when surrounded by a rich and phylogenetically diverse neighborhood of facilitated plant species (Brooker et al., 2008; Sortibrán et al., 2014), what can be partially influenced by the presence of CMNs in the soil (Sort- ibrán et al., unpublished results). Despite the potential of CMNs to influence the persistence of facilitative interactions, the specific mechanisms by which CMNs can promote facilitative interactions between adult plants are largely unknown.

Inter-connected plants can exchange water and nutrients along source-sink gradients (Bethlenfalvay et al., 1991; Frey and Schuepp, 1992; Simard et al., 1997, 2012; Egerton-Warburton et al., 2007; Querejeta et al., 2012). In the case of nitrogen, natural nitro- gen (N), source-sink gradients can result from the association of legume and non-legume species, as legumes in symbiosis with N2-fixing bacteria have access to atmospheric N, inaccessible to other plants (Dilworth et al., 2008). It is well known that legumes play an important role in structuring plant communities through plant facilitative interactions (Barnes and Archer, 1996; Flores and Jurado, 2003; Liphadzi and Reinhardt, 2006), and the N-transfer from a nurse legume to facilitated plants could be an ecologically relevant mechanism influencing plant facilitative interactions. The nitrogen transfer from legumes to non-legumes mediated by CMN has been largely studied sowing crop species in managed agro- ecosystems (Hamel et al., 1991; Hamel and Smith, 1991, 1992; Frey and Schuepp, 1992; Johansen and Jensen, 1996; He et al., 2004, 2005, 2006; Wichern et al., 2007; Teste et al., 2009; Laberge et al., 2011; Rasmussen et al., 2013; Chalk et al., 2014). However, far less research has been conducted in natural communities (but see He et al., 2006), and thus little is known about the role of nitro- gen transfer in more complex systems where multiple species can interact.

In this paper, we propose to test whether CMNs can promote nitrogen transfer between adult plants involved in long-lasting plant facilitative interactions. We selected an arid system in which we had previous experimental evidence that vegetation patches were originated by plant–plant facilitation processes (Castillo et al., 2010). We focus on species that were initially facilitated by a nurse species (i.e. growing within the same vegetation patch) and have survived until their adult stage (long-lasting facilitation interac- tions). We hypothesized that CMNs mediate N transfer from the nurse to adult facilitated plants. We selected 32 adult individuals of 6 facilitated species growing in 15 vegetation patches result- ing from the facilitation process triggered by the legume shrub Mimosa luisana. Following a balanced design, we treated a group of vegetation patches with fungicide, and another control group with water. Afterwards, we labeled the nurse plants with a 15N- tracer, and quantified the 15N transfer from the nurse plants to their facilitated plants. We expected greater N transfer to the facilitated plants in vegetation patches with intact CMNs (control), compared to individuals in vegetation patches treated with fungicide, and suggest that this can be a potential mechanism contributing to the persistence of long-lasting plant facilitative interactions.

Materials and methods

Study area

This experiment was conducted in the semiarid Valley of Zapotitlán (18◦211 N, 97◦281 W), a local basin of the biosphere reserve of Tehuacán-Cuicatlán Valley in the state of Puebla, Mexico. Aridity in this region is due to the rain shadow pro- duced 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 (June–August), and an annual mean temperature of 21 ◦C with rare frosts (García, 1988). Specifically, the study site is located 30 km south of Tehuacán city in a xeric shrubland dominated by the columnar cactus Neobuxbaumia tetetzo, and shrub species such as Mimosa luisana, Mascagnia seleriana, Ipomoea arborescens, Aeschynomene compacta, Caesalpinia melanadenia, Calliandra eryophylla, Zapoteca formosa, Senna wislizenii, Agave marmorata, Agave

155 macroacantha and Jat- ropha neopauciflora (Valiente-Banuet et al., 2000).

156

157 Plant–plant facilitation measurement

158

159 To verify that current community structure was governed by facilitation, in 2007 the
160 cover of perennial plants and bare ground was measured in four 1000 m2 plots. For each
161 species, the number of seedlings and saplings (<30 cm height) growing beneath plant
162 canopies and in bare ground areas was counted. Then, a contin- gency analysis was
163 conducted for all species together to compare the number of young individuals growing
164 beneath nurse plant canopies vs. bare ground (Table 1). Plant facilitative interactions
165 were confirmed to be driving the community structure resulting in

166 Table 1

167 Regeneration niche of species in the community. Species are considered facilitated if
168 the x2-test is significant, and the observed number of individuals (all species pooled)
169 recruiting under nurses is higher than expected by chance.

Number of species	Number of nurse species	% species facilitated	Number of ind. in open space	Number of ind. under nurse species	Total plant cover (%)	Bare ground cover (%)	x2-value	P-value
56	21	96	92	1237	71.1	28.9	367.5	<0.00001

170 Adapted from Valiente-Banuet and Verdú (2007). A significant greater amount of
171 individuals recruiting under nurse plants than in the bare ground (Table 1).
172 In this system, the legume M. luisana is a key nurse plant for most of the species in the
173 community, as 48 out of 56 of the species recorded recruit more frequently beneath it
174 than expected by chance. These include species of several functional groups – shrubs,
175 succulent plants such as Agave and cacti, perennial climb- ing vines, and perennial
176 herbs (Valiente-Banuet and Verdú, 2007). Most importantly, M. luisana is the only
177 nurse that can recruit in the bare ground and therefore it is responsible of the initial
178 formation of a vegetation patch (Sortibrán et al., 2014).

179

180 Vegetation patches as a proxy for plant–plant facilitation

The inability of most species to recruit in the open ground resulted in a patchy environment in which vegetation is clumped under the canopy of the nurse plants, usually M. luisana. Therefore, species growing within a patch (i.e. under the canopy of an adult individual of M. luisana) can be considered to be species facilitated by this nurse species. In addition, it was considered a long-lasting facilitation interaction when an adult facilitated and nurse plants persist within the same patch. The area occupied by a vegetation patch ranged from 1 to 5 m2, which corresponds to the vertical projection of the canopy of the nurse plant.

Field experiment

As the N isotopic composition of plant material depends on the age and type of tissue sampled (Dawson et al., 2002), we used species with leaves, which are produced in a relatively short amount of time. This constraint excluded cacti and agaves from the experimental design. We selected 32 adult individuals of 6 facil- itated species growing in 15 vegetation patches with an adult M. luisana. The six selected facilitated species and the nurse species are known to host arbuscular mycorrhizal fungi (Montesinos-Navarro et al., 2012b). Individuals were distributed in vegetation patches following a balanced design, so that we could treat the same num- ber of individuals per species with water (8 control patches) and the other half with fungicide (7 treated patches) (Table 2). These 15 vegetation patches were distributed within an area of 675 m2, with control and treated patches interspersed in space and at least 5–10 m apart from the nearest patch. These patches were used in a previous 2-year experiment using the fungicide Rovral 50% (Iprodione), which eliminates fungi very effectively, especially arbuscular mycorrhizal fungi, without affecting soil insects and bacteria (Gange et al., 1990; Ganade and Brown, 1997; Hernández- Dorrego and Mestre-Parés, 2010). During our previous experiment, Rovral reduced the percentage of root colonization by mycorrhi- zal fungi in the roots of M. luisana from 73.8% to 22% (Sortibrán et al., unpublished results). All the 7 patches treated with fungi- cide in this experiment had been previously treated with the same fungicide during the two previous years (at the

rate of 2.0 g/L of water, approx. 20 L per vegetation patch, at intervals of 3 weeks before the rainy season (six times) for 2 years), and 7 out of the 8 control patches had also been previously irrigated with the same amount of water. One more control patch was selected in 2013 to complete the balanced design, and similar results were observed in this patch compared to the other control patches. From May to July 2013 the fungicide and control treatments were restarted. Each 15 ± 5 days (four times), a dilution of 20–25 L of water with 2 g/L of the fungicide Rovral was applied in each of the treated patches and the same amount of water without fungicide was added to the control patches. During the application, the dilution was dispensed gradually using 3 or 4 canisters of 6 L, depending on the area of the vegetation patch. In order to prevent the leaching to other nearby vegetation patches, the dilution was dispensed into 3–5 holes, 5 cm deep each, dug in the ground of each patch. Considering that veg- etation patches ranged from 1 to 5 m2, this procedure ensured an even distribution of the fungicide throughout each patch.

Table 2 Post-labeling foliar 815N value (‰) for each nurse and facilitated plant species in each vegetation patch (after 15 days of the application of the 15 N tracer to the nurse plants). Control patches are named as Ctr-1 to Ctr-8 and Fungicide-treated patches as Fung-1 to Fung-7.

Patch	Nurse	Cathestecum brevifolium	Loeselia caerulea	Ruellia hirsutoglandulosa	Sanvitalia fruticosa	Siphonoglossa ramosa	Viguiera dentata
Ctr-1	−0.19		4.27	2.59			
Ctr-2	45.85	2.76	3.48		2.97	1.26	
Ctr-3	109.09						3.4
Ctr-4	122.81	24.71			3.8	14.68	
Ctr-5	123.25				6.84		
Ctr-6	123.73	4.69		8.55			
Ctr-7	265.19				5.51		
Ctr-8	1162.33	2.8	6.85				
Fung-1	5.63	1.23	0.54		4.74	2.15	
Fung-2	14.47	0.53	2.07				
Fung-3	23.58					2.63	
Fung-4	28.93		0.58	3.1	−1.71		2.65
Fung-5	54.52	1.35			−0.32		
Fung-6	143.92				4.51		
Fung-7	1159.04	1.76		2.3			

Nurse 15N labeling

At the beginning of August 2013, we labeled the nurse plants with urea enriched in 15N following the methodology proposed by Putz et al. (2011). We prepared urea solution by dissolving 4 g of urea at 98% 15N (Cambridge Isotope Laboratories, Inc.) in 2 L of water and 8 ml of surfactant. Each of the 15 M. luisana shrubs were systemically labeled by introducing individual branches in a 10 ml centrifuge tubes. In each M. luisana shrub, eight tubes were attached vertically to branches and sealed introducing the tube within a zip- lock plastic bag and sealing the bag with tape to reduce evaporation and avoid spillage. A total of 80 ml of 15N solution was provided to each M. luisana individual. Two weeks after the application, when most of the 15N solution had been absorbed, the labeled branches were cut to remove the bag without spillage. Approximately 1 g of fresh leaves was collected from the 32 individuals of the facili-

tated species (16 in control and 16 in treated patches) right before and 15 days after the application of the 15N labeling to the nurse plants (64 samples). Leaves were collected from different branches to represent the average foliar 15N content in the whole canopy of the plant. In the case of nurse plants, we ensure that we avoid col- lecting the leaves where the 15N solution was applied by cutting these branches as explained before.

Sample preparation and stable isotope analysis

Fresh leaves from all the facilitated and nurse plants (collected in the same individuals before and after the application of 15N to the nurse plant) were dried at 50 ◦C for 3 days and then ground to a fine powder. We encapsulated 3 mg of plant material into tin Capsules (8 × 5 mm Elementar Americas, Inc.) for nitrogen (815N) isotope analysis. The University of California, Davis Stable Isotopeatom%15N excess = atom%after− atom% before Facility (SIF) conducted 815N isotope analyses using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 continuous flow isotope ratio mass spectrometer. SIF used con- ventional delta (8) notation to report the relative difference of isotope ratios for samples (expressed in parts per thousand, ‰), and the international measurement standards N2 atmospheric gas (air) (3.677 × 10−3) for nitrogen (Coplen, 1994; IAEA, 2009). The precision of the 815N measurements was ±0.3 ‰.

Data analysis

In order to identify the significant sources of variation in foliar 815N in the facilitated plants, we used a Bayesian Generalized Linear Mixed Model. We tested for differences in foliar 815N as a func- tion of “Time” (before (pre-labeling) vs. after (post-labeling) nurse 15N-enrichment), “Treatment” (fungicide vs. control) and the inter- action term (Time × Treatment) using orthogonal contrasts and Gaussian distribution of errors. We ran two independent models; one for the nurse species (M. luisana), to confirm the success of the labeling with 15N, and another for the rest of species (facilitated species). In the latter case, we take into account that the 815N of the facilitated plants might not be independent if the samples were measured in: (a) the same individual, before and

after the application of the 15N to the nurse, (b) individuals of the same plant species, (c) plants growing in the same patch, (d) plants facilitated by a nurse with a given 15N enrichment. To do so, we considered "facil- itated individual plant", "facilitated species", "patch" and "nurse 815N value" as random factors in the model. The models were run with the help of MCMC techniques as implemented in the MCM- Cglmm package for R (Hadfield, 2010; R Development Core Team, 2011). We used the default priors and ran 2000000 MCMC iterations sampled each 1000 with a burn-in period of 25%. Convergence was assessed by visual inspection and it was checked that autocor- relation between successive stored iterations was lower than 0.1. The statistical significance of the factors in the model was estimated by calculating the 95% credible interval (CI) of their posterior disTribution and checking afterwards that zero was not included in that interval. If CMN play a role in Ntransfer from the nurse plant to the facilitated species, we expect a significant interaction term between "Time" and "Treatment". Specifically, we expect a greater difference between the post-labeling–pre-labeling 815N (i.e. increment in 815N), in the facilitated plants growing in patches with intact CMNs (control patches) than in patches where CMNs had been reduced (patches treated with fungicide).

2.8. Estimates of N transfer

In order to quantify the average 15N transfer between plants, only cases in which the nurse enrichment was unequivocal (i.e. nurse increment in 815N > 10‰) were selected. To estimate the per- centage of 15N tracer transferred from the nurse to the facilitated plant, sample 815N values were converted to absolute isotope ratio (R) as in Teste et al. (2009):

$$R_{sample} = [(815N/1000) + 1] \times R_{standard}$$

The percentage contribution of the heavy isotope to the total num- ber of atoms of that element in the sample (atom%) was calculated following Dawson et al. (2002):

$$atom\%15N = 100 \times (R_{sample}/(R_{sample} + 1))$$

The background atom% values were subtracted from the sample values after the application of the 15N to calculate the atom% excess (Teste et al., 2009):

Finally, following Tomm et al. (1994) the percentage of the 15N tracer in the receiver derived from N transfer from the donor (% NDFT) was calculated as:

% NDFT = (atom%15N excessreceiver/atom%15N excessdonor) × 100

Results

The 15N labeling of the nurse plants was effective, as they signif- icantly increased their foliar 815N values after 15 days of the tracer application, from 1.25 ± 0.30‰ to 225.48 ± 99.69‰ (mean ± SE); post mean = 111.59 [5.44, 213.54]. All the 15 nurse plants increased their foliar 815N after the application of the tracer, and all but two showed a 815N increment (i.e. difference between post- labeling–pre-labeling levels of 815N) greater than 10‰. One poorly 15N-enriched nurse plant was in a control patch and the other in a fungicide-treated patch. There were no overall differences between the average foliar 815N values of nurse plants grow- ing in control and fungicide-treated patches (post mean = −10.46 [−109.951, 97.03]), and nurse plants did not show a significant Time × Treatment interaction effect (post mean = −11.01 [−126.04, 80.60]). However, considering facilitated species, there was a signifi- cant Time × Treatment interaction effect (Table 3). As expected, facilitated adult plants showed a greater increment (post- labeling–pre-labeling) in foliar 815N values in control patches than in patches treated with fungicide (Time × Treatment interaction effect) (Table 3 & Fig. 1)

Table 3

Results of the Bayesian Generalized Linear Mixed Model explaining the variation in 815N in the facilitated plants as a function of Time (before vs. after nurse 15 N labeling), Treatment (fungicide vs. control) and the interaction between them. Statistically significant factors (*) in the model were those whose 95% credible interval of their posterior distribution did not include zero.

	Post mean	Lower CI-95%	Upper CI-95%
(Intercept)	3.36*	2.41	4.26
Time (before vs. after)	0.62	−0.19	1.49
Treatment (control vs. fungicide)	−1.35*	−2.27	−0.44
Time × Treatment	−0.85*	−1.73	−0.04

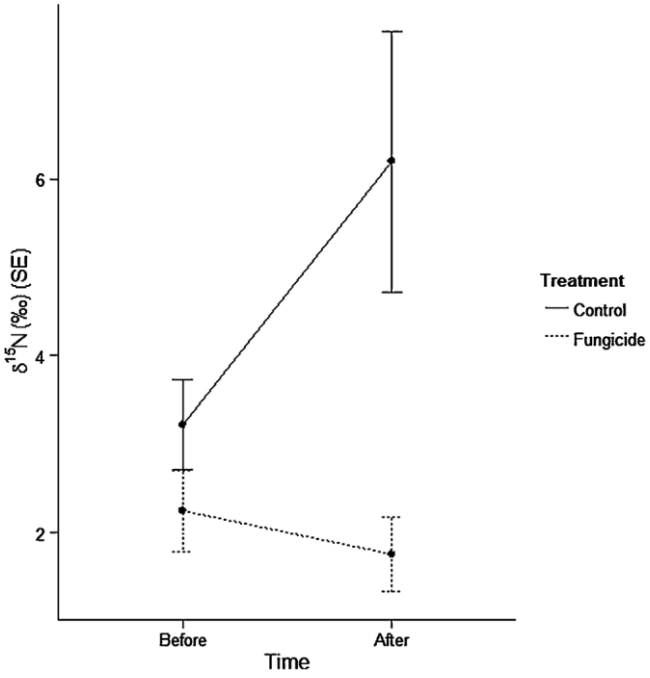

Fig. 1. Mean (standard error) foliar 815N of facilitated plants before and after the 15 N labeling of their nurse plants in control and fungicide-treated patches.

thus implying a greater N transfer from the nurse plant to the facilitated plants in patches where the CMNs were intact.In order to estimate the percentage of 15N transfer, we considered the seven control patches in which the nurse plants showed an enrichment >10‰. The mean percentage of the 15N tracer trans- ferred from the nurse to the facilitated plants was 2.64 ± 1.49% NDFT. Fifteen days after the application of the 15N tracer, some facilitated plants showed foliar 815N (Table 2) values which are unlikely to be due to natural abundance fluctuations of the nitro- gen isotopic composition of the plants. This provides unequivocal evidence of actual transfer of 15N between the nurse and the facili- tated species. That was the case for one individual of the perennial grass Cathestecum brevifolium that received 18.9% NDFT. Even with- out considering this exceptionally high value, the mean percentage of the 15N transferred from the nurse to the facilitated plants was still 1.38± 0.88% NDFT.

Discussion

Elucidating the mechanisms that contribute to the persistence of plant facilitative associations in the long term can improve our understanding of plant co-existence and maintenance of biodiver- sity. Plant facilitative associations will be prone to last if there is a persistent benefit between the adult plants involved. We show that nitrogen can be transferred from the nurse to adult facilitated plants through CMNs, suggesting a mechanism that can contribute to the long-term persistence of plant facilitative associations.

According to our expectations, N transfer is reduced in patches treated with fungicide, which demonstrates that N transfer is to some extent mediated by CMNs. However, our experiment does not tease apart the preferential pathway of N transfer. Several previ- ously proposed mechanisms can result in the observed pattern. For example, (a) nitrogen can be transported from one plant to another directly through hyphal links connecting the roots of both plants (Bethlenfalvay et al., 1991; Frey and Schuepp, 1992, 1993; Johansen and Jensen, 1996), or (b) nitrogen can be released to the rhizosphere by the nurse plant as root exudates, and then be taken up by the fungal hyphae harbored in the facilitated plants roots (Marschner and Dell, 1994; Smith and Read, 2008). Independently of the spe- cific preferential pathway, our experiment shows that in the short term, the N transfer pathway mediated by mycorrhizal fungi is more effective than the “nurse root-soil-facilitated root” pathway (via root exudates by the nurse plant, without fungal mediation).

The detected amount of 15N transfer from the nurse to the facilitated species must be considered as a conservative estimate, especially taking into account the short time allowed for the trans- fer to occur. Although we find a significant N transfer in just 15 days, several studies show that N transfer from the donor can be accu- mulated over longer periods, and can amount to up to 40% of total N in the facilitated plant over several months (Høgh-Jensen and Schjoerring, 1997; He et al., 2006; Rasmussen et al., 2007). In addi- tion, the fact that multiple pulses of tracer application increase the amount of N transfer supports its cumulative nature (Gylfadóttir et al., 2007), potentially resulting in large amounts of N transferred in the long-term. Nevertheless,

386 although comparing 15N transfer estimates requires caution due to different
387 methodologies, scales and incon- sistencies in terminology, our results match most of
388 the published estimates, generally showing low transfer values (<10% and often <1%),
389 with just a few exceptions showing higher values (up to 50%) (He et al., 2009; Chalk et
390 al., 2014). Even in controlled microcosm experiments, where donor and receptor plants
391 grow in compart- ments separated by root-excluding mesh that allows mycorrhizal
392 hyphal passage, the amounts of 15N transfer reported are similar to those observed in
393 this study (less than 4% (Frey and Schuepp, 1992); 0.7–2.5% (Jalonen et al., 2009)). It
394 is not clear how these relatively small amounts of N transfer can influence adult plants
395 fitness. How- ever, it is reasonable to presume that interplant N transfer can be a
396 relevant resource for facilitated plants, especially in semi-arid envi- ronments where soil
397 N is a limiting factor and N-fixing legumes are key nurse species structuring plant
398 communities through facilita- tion (Flores and Jurado, 2003; Bashan et al., 2009; Muro-
399 Pérez et al., 2012). Remarkably, legumes in agro-ecosystems may contribute up to 270–
400 550 kg N ha−1 year−1 (Sanginga et al., 1994; Jayasundara et al., 1997; Dulormne et al.,
401 2003), mainly due to symbiotic N2 fix- ation, which can account for 30–90% of their
402 total N (Giller, 2001). Several field studies have shown that non-legume crops cultivated
403 with legumes may obtain a substantial proportion of their N from the latter (Høgh-
404 Jensen and Schjoerring, 2000; Snoeck et al., 2000; Sierra and Nygren, 2006; Daudin
405 and Sierra, 2008), although little is known about the magnitude of this process in natural
406 ecosystems.
407 Over long periods of time, the accumulation of small amounts of nutrient transfer can
408 have ecological consequences for both the nurse and facilitated species involved in
409 persistent facilita- tive interactions. Previous experiments show that the performance of
410 adult plants of the nurse species M. luisana (seed production) is not affected by a two-
411 year fungicide treatment when growing alone, but decreased when growing associated
412 to their facilitated plants (Sortibrán et al., unpublished results). This indicates that the
413 reduction in the performance of M. luisana is not mediated by the fungicide effects on
414 its own fungal associates, but instead by the fungicide effects on the CMNs connecting
415 M. luisana to its facili- tated plants. Our results show that CMNs can mediate N transfer
416 between adult facilitated plants, suggesting that nutrient transfer through CMNs might

be a potential mechanism allowing persistent benefits for adult facilitated plants. It is intriguing which evolutionary processes could explain the N transfer from plant to plant or even from mycorrhizal fungi to the facilitated plant, considering that mycorrhizal fungi have a much higher requirement for N than plants (optimal C:N ratio for plant leaf tissue 33:1, for fungal hyphae 10:1; Allen et al., 2003). It has been suggested that the plant-mycorrhizal symbiosis is based on a system of reciprocal rewards that provides both partners with a certain degree of control over the symbiosis by investing more resources on partners that provide more benefits (Kiers et al., 2011). Under this scenario, mycorrhizal fungi might benefit from redis- tributing nitrogen among their plant partners along source-sink gradients (e.g. from legumes to non-legumes) to ensure the main- tenance of multiple sources of carbon, while plants connected by CMNs could mutually benefit from exchanging their less limiting (or surplus) nutrients along source-sink gradients. Nevertheless, much controversy remains regarding the mechanisms that actu- ally govern resource exchange in the plant-mycorrhizal symbiosis (Walder and van der Heijden, 2015).

Conclusions

We show that N transfer between adult plants is promoted by mycorrhizal networks. It is known that adult-nurse plants bene- fit from growing with adult-facilitated plants in our study system (Sortibrán et al., 2014). However, a benefit for the adult-facilitated plants may also favor the long-term persistence of the facilitative interaction. Our results suggest that inter-plant N transfer medi- ated by CMNs can be a mechanism by which adult facilitated plants continue receiving benefits from their nurse. Further research on the fitness consequences of nutrient transfer between adult plants will be necessary to improve our understanding of the evolutionary implications of facilitative interactions in structuring plant commu- nities and maintaining plant diversity.

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