Nurse plants transfer more nitrogen to distantly related species

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Abstract. Plant facilitative interactions enhance co-occurrence between distant relatives, partly due to limited overlap in resource requirements. We propose a different mechanism for the coexistence of distant relatives based on positive interactions of nutrient sharing. Nutrients move between plants following source–sink gradients driven by plant traits that allow these gradients to establish. Specifically, nitrogen (N) concentration gradients can arise from variation in leaf N content across plants species. As many ecologically relevant traits, we hypothesize that leaf N content is phylogenetically conserved and can result in N gradients promoting N transfer among distant relatives. In a Mexican desert community governed by facilitation, we labelled nurse plants (*Mimosa luisana*) with ^{15}N and measured its transfer to 14 other species in the community, spanning the range of phylogenetic distances to the nurse plant. Nurses established steeper N source–sink gradients with distant relatives, increasing ^{15}N transfer toward these species. Nutrient sharing may provide long-term benefits to facilitated plants and may be an overlooked mechanism maintaining coexistence and increasing the phylogenetic diversity of plant communities.

Key words: coexistence; ^{15}N; nitrogen transfer; nutrient sharing; phylogenetic distance; plant–plant interactions.

Introduction

Coexistence theory is framed within the competition paradigm and focuses particularly on species whose coexistence has been traditionally explained by mechanisms stabilizing niche differences or equalizing the fitness of competitive species, ultimately preventing competitive exclusion (Chesson 2000, Mayfield and Levine 2010, Godoy et al. 2014). Recently, there have been attempts to include plant facilitative interactions within coexistence theory, including mechanisms such as realized niche expansion and prevention of local extinction (Bruno et al. 2003, Soliveres et al. 2015, Valladares et al. 2015, Bulleri et al. 2016). Plant facilitative interactions are ubiquitous in many ecosystems (Gómez-Aparicio et al. 2004, Callaway 2007, Brooker et al. 2008) and are important drivers structuring plant communities (Brooker 2006). Facilitative interactions take place when one species (i.e., nurse species) benefits another (i.e., facilitated species) without resulting in any damage to the former or even resulting in a mutual benefit for both species (Callaway 2007, Sortibrán et al. 2014). Some facilitative interactions shift to competition during the ontogeny of the facilitated plant (Schöb et al.

2014), while others persist over time resulting in adult plant coexistence (Valiente-Banuet and Verdú 2008). Persistent facilitative interactions tend to occur between distantly related plant species, therefore shaping the phylogenetic structure of plant communities (Valiente-Banuet et al. 2006, Valiente-Banuet and Verdú 2007, 2013). The mechanisms proposed to maintain these positive interactions through time have also been framed within the competition paradigm, suggesting stabilizing niche differences between the species involved (Pausas and Verdú 2010). Distantly related organisms tend to have little resemblance to each other in terms of physiology, life strategies, or functional traits (Blomberg et al. 2003), resulting in niche segregation, which reduces interspecific competition and enhances coexistence (Webb et al. 2002, Pausas and Verdú 2010). However, the phylogenetic pattern of plant facilitative interactions can also be attributed to a different coexistence mechanism not based on competition but on positive interactions. Differences in functional traits that are evolutionarily conserved can also result in functional complementarity between distant relatives. Phylogenetic patterns in plant nutrient contents can thus result in positive interactions of nutrient sharing between distantly related plants.

In N-limited systems, legumes can increase the N available in soil through their capacity to establish

symbiotic associations with N_2-fixing microorganisms (Dilworth et al. 2008). Many plant species in semiarid systems require the micro-environmental conditions provided by legumes to establish (Barnes and Archer 1996, Flores and Jurado 2003, Liphadzi and Reinhardt 2006, Valiente-Banuet and Verdú 2007), and these facilitative interactions can take place through several mechanisms including stress amelioration, increased habitat heterogeneity, or sharing mutualisms (Callaway 2007, Gómez-Aparicio 2009, Michalet et al. 2011). Legumes in particular can make a considerable contribution to soil fertility by providing up to 270–550 kg $N \cdot ha^{-1} \cdot yr^{-1}$ through root exudates or litter deposition (Sanginga et al. 1994; Jayasundara et al. 1997; Dulormne et al. 2003).

Neighboring plants can exchange nutrients through mycorrhizal fungi present in their shared rhizosphere (Hauggaard-Nielsen and Jensen 2005, Van der Heijden and Horton 2009), resulting in fungal-mediated N transfer along source–sink N gradients (i.e., from the N-rich plant to the N-limited plant; Bethlenfalvay et al. 1991, Frey and Schüepp 1993). Species-specific traits influencing N content can result in source–sink N gradients among coexisting plants, which may promote nutrient sharing between distantly related species if traits influencing N content are phylogenetically conserved. Leaf N content is high in clades harboring N-fixing plants when this trait is analyzed across the entire phylogeny of angiosperms (Cornwell et al. 2014), but N content can also be phylogenetically conserved independently of this (Stock and Verboom 2012, Yang et al. 2015). Although there is still limited evidence to assess whether closely related species have similar N content, two types of evidence support the idea that the N content of plant species can be phylogenetically conserved. Firstly, foliar nutrient content has been shown to be evolutionarily conserved within clades (Nakadai et al. 2014, Oguro and Sakai 2014), and secondly, the capacity for symbiotic N_2-fixation is now known to be shared by a monophyletic clade in angiosperms (Werner et al. 2014), resulting in similar foliar N concentrations among closely related species (i.e., high for nodulating species and low for non-nodulating species; Högberg 1997).

In this study, we hypothesize that (1) foliar N concentration in facilitated plant species will decrease with increasing phylogenetic distance to the nodulating nurse species thus creating steeper N source–sink gradients and (2) N transfer from a nurse plant will increase toward its most distantly related facilitated species. We selected 14 coexisting species in a semiarid Mexican plant community that are facilitated by the N-fixing *Mimosa luisana* encompassing the range of phylogenetic distances to this nurse. We labelled the nurse *M. luisana* with a ^{15}N-enriched tracer and measured the increase in the foliar $\delta^{15}N$ values of the facilitated species after 15 d as a proxy of the N transferred from the nurse. With this approach, we test whether inter-plant N transfer is more likely to occur between distantly related species,

providing a new mechanism explaining plant persistence among distantly related species.

METHODS

Study site and species selection

The study site was located in the Valley of Zapotitlán (18°21′ N, 97°28′ W) in the state of Puebla, Mexico. It is a semiarid region with mean annual temperature of 21°C, rare frosts, and average annual rainfall of 380 mm, most of which falls during the summer months (June–August; García 1973). The vegetation is a xeric shrubland community dominated by the columnar cactus *Neobuxbaumia tetetzo*, with plant taxa belonging to the families Fabaceae, Malpighiaceae, Verbenaceae, and Asteraceae among others (Valiente-Banuet et al. 2000). Most plant species in this system are unable to recruit in bare ground and require facilitation by the nurse *Mimosa luisana* to establish (Verdú and Valiente-Banuet 2008). On the contrary, plants of *M. luisana* rarely establish under a conspecific, usually resulting in vegetation patches containing a single *M. luisana* plant. Different types of benefits are provided by *M. luisana* to the facilitated species. The shade of this deciduous spiny shrub, which can reach heights up to 2–3 m and a maximum diameter of 1.5–3.0 m, can reduce soil water evaporation, thus increasing moisture availability underneath its canopy. Besides amelioration of abiotic stress, there is also evidence of biotic factors driving facilitation in this system (Castillo et al. 2010, Montesinos-Navarro et al. 2012a, 2016). The establishment, recruitment, and growth of some species depends on the phylogenetic diversity of their neighborhood, suggesting that indirect effects among plants and/or their associated microbiota can shape the outcome of plant facilitative interactions (Castillo et al. 2010). *Mimosa luisana* harbors the greatest richness of arbuscular mycorrhizal fungi (AMF) in this community, and increasing AMF richness in the shared rhizosphere can promote plant facilitative interactions (Montesinos-Navarro et al. 2012a, b). Finally, *M. luisana* can also benefit facilitated species through N fixation, which can be especially relevant in N-limited soils. At our study site, the total soil N content in bare ground (without any vegetation) is 0.14% ± 0.01% (Sortibrán et al. 2014), which is relatively low considering that soil N content across biomes ranges from 0.01% to 3% globally (Ordoñez et al. 2009). In addition to *M. luisana*, there are other N-fixing legumes at the study site that can also provide N to the shared rhizosphere, including *Prosopis laevigata* and *Acacia coulteri*. These legumes facilitate other plant species in the community, but *M. luisana* is by far the highest quality nurse plant as it nurses three times more facilitated plants than any of these other legumes; it is also much more abundant (Valiente-Banuet and Verdú 2007). There is evidence that facilitative interactions drive a patchy vegetation distribution of the vegetation in this environment (Valiente-Banuet and Verdú 2007,

2008, Castillo et al. 2010, Verdú et al. 2010, Verdú and Valiente-Banuet 2011). Within these vegetation patches, recent studies have shown that the nurse plant can transfer N to different species of facilitated plants through common mycorrhizal networks (Montesinos-Navarro et al. 2016).

To test our hypotheses, we selected 14 facilitated species that cover the range of phylogenetic distances between *M. luisana* and all species in the community (from 0 million years (Myr; i.e., an individual of *M. luisana* growing in close proximity to another *M. luisana*) to 322 Myr (*Bouteloua gracilis*). Therefore, our sampling unit (i.e., replicate) was the phylogenetic distance between *M. luisana* and a facilitated species, and thus we focused our effort on selecting as many phylogenetic distances as possible, rather than multiple individuals of the facilitated species studied. Nevertheless, we also show that our approach is robust to the consideration of intra-specific variation in N transfer by repeating the analyses using previously published data of intra-specific variation for several plant species from the same study system (Montesinos-Navarro et al. 2016) (see details in *Statistical analyses* and Appendices S1 and S2). We focused on facilitated species having leaves with a relatively fast turnover to ensure a similar tissue age across species since plant $\delta^{15}N$ natural abundance depends on tissue age and type (Dawson et al. 2002). This constraint excluded Cactaceae and Agavaceae from the experiment. The selected facilitated species were: *M. luisana*, *Zapoteca formosa*, *A. coulteri*, *P. laevigata*, *Senna wislizenii*, *Aeschynomene compacta*, *Dalea carthagenensis*, *Echinopterys eglandulosa*, *Cardiospermum halicacabum*, *Cissus sicyoides*, *Thompsonella minutiflora*, *Loeselia caerulea*, *Viguiera dentata*, and *B. gracilis*. We subsequently selected one individual per facilitated species growing underneath an adult nurse plant (*M. luisana*). The 14 facilitated plants were distributed in seven different vegetation patches of 2–3 m in diameter

located within an area of 250 × 250 m (Tables 1 and 2). All patches had a single nurse but a different number of facilitated plants were selected (three patches had one of the selected facilitated plants, two patches had two plants, and the other two patches had three and four plants, respectively).

Phylogenetic distances

The phylogenetic distance matrix among all study plant species was obtained from a community phylogeny generated with the Phylomatic algorithm implemented in Phylocom 4.2 (Webb et al. 2008). This program produced a community phylogeny by matching the family names of our 14 species with those contained in a backbone phylogeny, the R20120829 megatree assembled following the Angiosperm Phylogeny Group III (Stevens 2005). The tree was further resolved and calibrated with age estimates from Wikström et al. (2001) and Zanne et al. (2014) plus all available ages obtained from chronograms and phylogenies published for specific families and genera. We resolved the phylogenetic relationships at the species level of the Fabaceae family based on published phylogenies of Simon et al. (2009). Finally, we dated nodes of the Poaceae and Malpighiaceae families based on Davis et al. (2005) and Magallón et al. (2015). For the undated nodes, we adjusted branch lengths with the help of BEAST (Bayesian Evolutionary Analysis Sampling Trees; Drummond and Rambaut 2007) and the PolytomyResolver script (Kuhn et al. 2011). This branch length adjustment procedure is based on an evolutionary, birth–death model, which has been shown to be more realistic than traditional non-model-based approaches (Kuhn et al. 2011). In order to account for the uncertainty in node age estimates using evolutionary models, we repeated all analyses with 100 different phylogenies resulting from the application of this model. The phylogenetic distances between *M. luisana* and the facilitated species were obtained using the ape package (Paradis et al. 2004) and we tested for a phylogenetic signal of leaf N content using Blomberg's *K* statistic (Blomberg et al. 2003) implemented in the function phylosig within the phytools package in R version 3.2.2. In order to ensure that our results were robust despite the uncertainty in the estimation of divergence times among taxa, we repeated all analyses using different estimates of the phylogenetic distance between the 14 facilitated species and *M. luisana* as reported in the database TimeTree (http://www.timetree.org/). This database compiles expert-knowledge at molecular time estimates of divergence among taxa. We obtained similar results in both cases and therefore, only the analyses based on BEAST are presented.

TABLE 1. ^{15}N-enrichment in individuals of the nurse species *Mimosa luisana* labeled with ^{15}N-enriched urea.

Patch	$\delta^{15}N$ before (‰)	N_{tot} before (µg/mg)	$\delta^{15}N$ after (‰)	N_{tot} after (µg/mg)
A	−0.59	33.97	133.11	30.20
B	−0.16	32.42	212.64	28.95
C	−1.31	31.60	109.09	30.23
D	−0.60	28.38	1,879.54	25.51
E	1.67	34.16	12.02	28.62
F	0.65	30.70	122.81	30.54
G	0.70	34.88	14.47	29.07
Average (SE)	0.05 (0.38)	32.30 (0.86)	354.81 (255)	29.02 (0.64)

Note: The vegetation patch code in which each labeled nurse plant was located, and the foliar $\delta^{15}N$ and N concentration (N_{tot}) measured before and after the application of the ^{15}N-enriched tracer are shown.

^{15}N transfer experiment

All nurse plants in the seven vegetation patches were successfully labelled by foliar immersion in a ^{15}N-enriched

TABLE 2. The ^{15}N-enrichment of facilitated species.

Facilitated species	Family	P	δ^{15}N before (‰)	N$_{tot}$ before (mg/g)	δ^{15}N after (‰)	N$_{tot}$ after (mg/g)
Mimosa luisana (0)	Fabaceae	F	2.15	34.85	2.23	31.17
Zapoteca formosa (62)	Fabaceae	C	2.07	33.14	3.20	30.27
Acacia coulteri (86)	Fabaceae	A	−0.57	36.13	−0.22	32.86
Prosopis laevigata (92)	Fabaceae	A	2.41	39.95	2.42	37.24
Senna wislizenii (122)	Fabaceae	A	3.69	34.02	4.08	33.85
Aeschynomene compacta (130)	Fabaceae	B	−0.76	28.99	−0.54	24.46
Dalea carthagenensis (132)	Fabaceae	D	−0.36	27.48	0.12	27.13
Echinopterys eglandulosa (250)	Malpighiaceae	D	2.04	46.68	1.26	42.09
Cardiospermum halicacabum (264)	Sapindaceae	C	1.06	35.15	1.97	30.75
Cissus sicyoides (277)	Vitaceae	C	2.41	35.24	5.61	35.27
Thompsonella minutiflora (291)	Crassulaceae	G	1.75	15.00	3.14	14.53
Loeselia caerulea (309)	Polemoniaceae	E	1.77	6.30	4.25	10.73
Viguiera dentata (309)	Asteraceae	C	2.35	31.78	3.4	28.77
Bouteloua gracilis (322)	Poaceae	B	−1.04	10.16	1.33	10.50

Notes: The vegetation patch code where each facilitated plant was located (P), and the foliar δ^{15}N and N concentration (N$_{tot}$) measured before and after the application of the ^{15}N-enriched tracer to its nurse. The mean phylogenetic distance to *M. luisana* (Myr), averaged across 100 phylogenetic trees is provided in parentheses.

urea solution (Putz et al. 2011) prepared by dissolving 4 g urea (98% ^{15}N; Cambridge Isotope Laboratories, (Sercon Ltd., Cheshire, UK)) and 8 mL surfactant in 2 L water. Eight tubes of this solution (10 mL per tube) were systemically applied to each individual of *M. luisana* by immersing one branch in each tube. Tubes were attached vertically to branches, placed within a press seal plastic bag and sealed with duct tape to reduce evaporation and avoid spillage (Ledgard et al. 1985). Each *M. luisana* individual had access to 80 mL of ^{15}N solution during 2 weeks. After 15 d, labelled branches were cut to remove the bag without spillage. The seven *M. luisana* nurse plants and the 14 individuals of the facilitated species were sampled immediately before and 15 d after the ^{15}N labelling of *M. luisana* to calculate N transfer. Approximately 1 g of fresh leaves was collected from each plant at each sampling point. Leaves were collected from different branches to represent the average foliar N concentration in the whole canopy of the plant. In the case of nurses, the leaves sampled were different from those to which the ^{15}N-enriched solution was applied.

Sample preparation and stable isotope analysis

The leaf material from each target plant was dried at 50°C for 3 d and ground to a fine powder. Afterward, 3 mg of leaf material per plant was weighed and encapsulated into tin capsules (8 × 5 mm; Elementar Americas, Mt Laurel, New Jersey, USA) for N isotope analysis (δ^{15}N) and total N concentration measurements (hereafter N$_{tot}$). In order to estimate the repeatability of the measurements, 7% of the sampled were analyzed twice. The δ^{15}N analyses were conducted at the University of California, Davis Stable Isotope Facility (SIF), using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 continuous flow isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Conventional delta (δ) notation is used to report the isotope ratios of foliar samples (expressed in parts per thousand, ‰) relative to international standards (atmospheric N$_2$; Coplen 1994). Analytical error for δ^{15}N measurements is ±0.3‰.

Calculation of the variables

To test whether inter-plant N transfer increases with steeper N source–sink gradients, we calculated two variables: N transference and N$_{tot}$ gradient. The amount of N transference (i.e., ^{15}N-tracer transferred from the nurse to each facilitated species) was estimated as the increase in foliar δ^{15}N observed in the facilitated species 15 d after the ^{15}N labeling of their nurse (δ^{15}N$_{after}$ − δ^{15}N$_{before}$). The N$_{tot}$ gradient was estimated as the difference in foliar N$_{tot}$ between the nurse and each facilitated plant (Nurse$_{N_{tot}}$ − Facilit$_{N_{tot}}$).

Statistical analyses

We used three Bayesian generalized linear models (GLM) to test whether (a) foliar N$_{tot}$ in facilitated species decreases with their phylogenetic distance to *M. luisana*, (b) inter-plant N transfer increases with steeper source–sink gradients, and (c) the amount of N transferred to the facilitated species increases with the phylogenetic distance to *M. luisana*. We used as dependent variable Facilit$_{N_{tot}}$ in model a, and the amount of N transference in the models b and c; and as fixed explanatory variables, the phylogenetic distance to *M. luisana* in models a and c, and N$_{tot}$ gradient in model b. The Bayesian approach allows the incorporation of the variance in the node ages estimated by BEAST. To do so, we ran a GLM model for each of

the 100 randomly selected phylogenies generated by BEAST. Then we drew 1000 random samples of the Markov chains iterations across these 100 models to obtain an approximation to the posterior distribution of the estimate (i.e., the slope of the relationship between the dependent and the explanatory variable). We ran the three models using physical distance to the nurse and nurse enrichment in ^{15}N as random factors. This indirectly accounts for the spatial distribution of facilitated species in the seven patches as facilitated individuals sharing a vegetation patch also share a nurse (with a specific ^{15}N-enrichment). We used the default priors with 250,000 MCMC (Markov chain Monte Carlo) iterations sampled every 100 and discarded a burn-in period of 50,000. We performed additional analyses to account for intraspecific variation in the amount of ^{15}N transferred to the facilitated plants. These analyses were based on published data from a parallel experiment conducted in the same system (Montesinos-Navarro et al. 2016), where intraspecific variation could be calculated for a subset of the facilitated species. These analyses, which accounted for intraspecific variation, yielded similar results to those considering one individual per species. Thus, we only report and discuss the latter in the main text with further details about the former provided in Appendices S1 and S2.

In all cases convergence was assessed by visual inspection and the settings were chosen to maintain the autocorrelation between successive stored iterations below 0.1 (Hadfield 2010). For each of the three tests we drew a histogram showing the posterior distribution of the estimates of the slope. In addition, we used the posterior distribution of the estimates of the slope to provide the probability that the estimate is higher or lower than zero depending on the expectations for each specific test, and we drew a histogram with the estimates in order to facilitate the assessment of which proportion resulted in a positive slope (greater than 0). All the analyses were performed using the MCMCglmm package (Hadfield 2010) for R software version 3.2.2.

RESULTS

The amount of ^{15}N tracer transferred from the nurse plant to the facilitated species was not significantly correlated with competitive effects driven by either the total number of species ($N = 14$, df $= 12$, $t = 1.49$, P value $= 0.16$), the total number of individuals ($N = 14$, df $= 12$, $t = 0.66$, P value $= 0.52$) or the number of selected plants growing in its patch ($N = 14$, df $= 12$, $t = 0.21$, P value $= 0.84$), so these variables were not included in further analyses. Both the physical distance to the nurse and nurse enrichment in ^{15}N were accounted for in the analyses including them as random factors. We found that foliar N_{tot} of facilitated species was phylogenetically conserved (K-statistic significantly higher than 0: $K = 1.17$ P value < 0.05) and decreased with phylogenetic distance to the nurse *M. luisana* (posterior mean estimate of the slope $= -0.050$; Probability of the slope being lower than $0 = 95.6\%$, Fig. 1), thus generating steeper N_{tot} gradients in distantly related facilitated plants. At the beginning of the experiment, the average (mean $\pm$ SE) foliar N_{tot} concentration in the *M. luisana* plants ($Nurse_{N_{tot}}$) was 32.11 ± 0.54 mg/g, whereas foliar

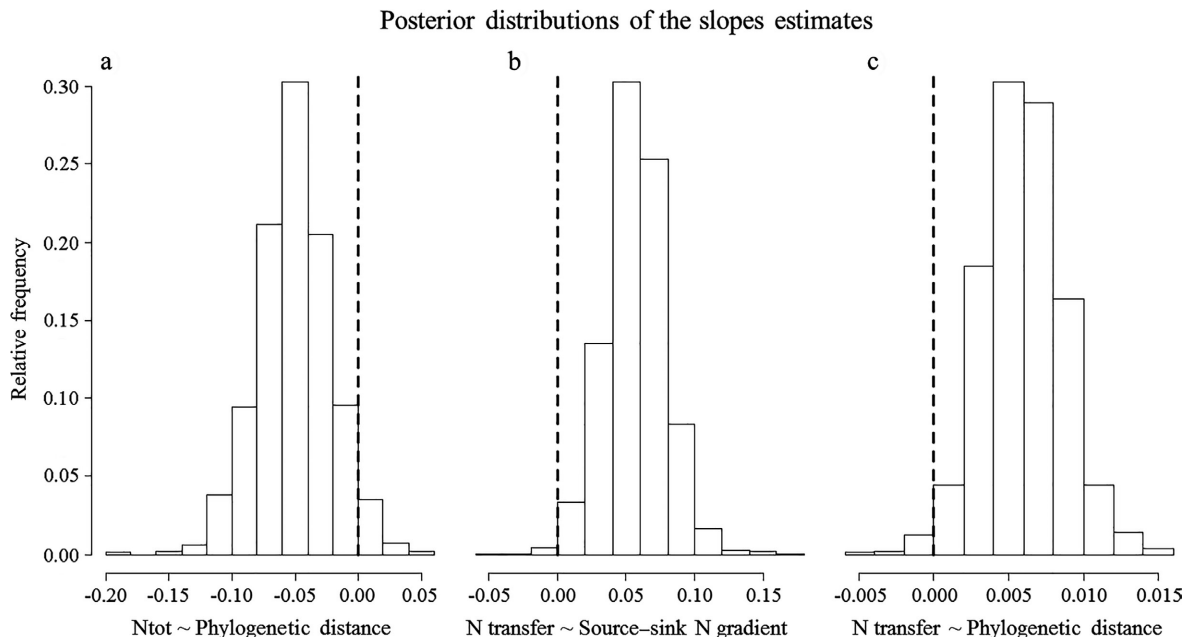Posterior distributions of the slopes estimates

FIG. 1. The posterior distribution of the estimates of the slopes for each of the three hypothesis tested: (a) a negative slope between the foliar N concentration (N_{tot}) and the phylogenetic distance to *Mimosa luisana*, (b) a positive slope between N transfer ($\delta^{15}N_{After} - \delta^{15}N_{Before}$) and N source–sink gradients ($Nurse_{N_{tot}} - Facilit_{N_{tot}}$), and (c) a positive slope between N transfer and the phylogenetic distance to the nurse.
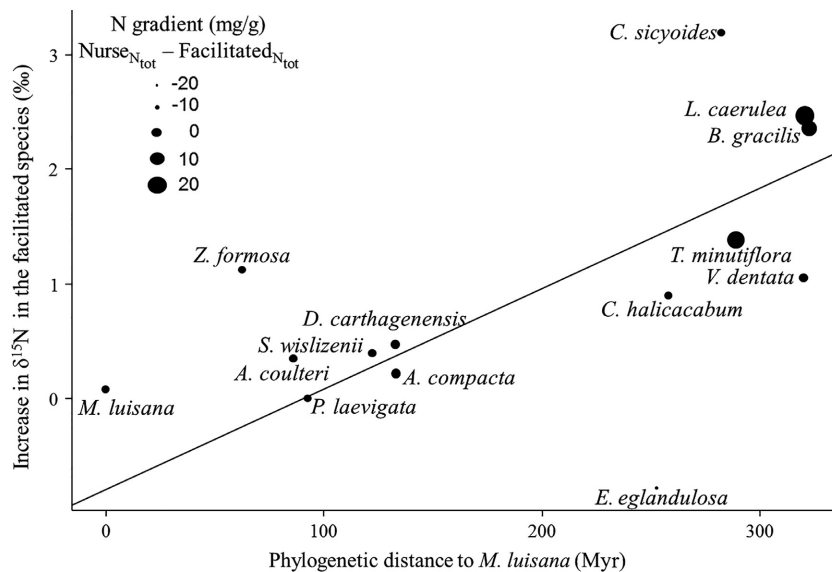
Fig. 2. Increase in ^{15}N transfer from the nurse to distantly related facilitated species following a source–sink gradient. For each facilitated species, the nitrogen transfer from the nurse is estimated as the increase in foliar $\delta^{15}N$ values (‰) 15 d after the ^{15}N labeling of their nurse ($\delta^{15}N_{After} - \delta^{15}N_{Before}$). The size of each point represents the N_{tot} concentration (mg/g) gradient between the nurse and the facilitated plant (foliar N concentration of the nurse − foliar N concentration of the facilitated) at the beginning of the experiment. Phylogenetic distance is expressed in millions of years (Myr). Species names are spelled out in Table 2.

N_{tot} concentration in the 14 facilitated species ($Facilit_{N_{tot}}$) ranged from 6.29 mg/g (*Loeselia caerulea*) to 46.68 mg/g (*Echinopterys eglandulosa*). On average, the N_{tot} gradient between each nurse and its facilitated plant species ($Nurse_{N_{tot}} - Facilit_{N_{tot}}$) was 3.48 ± 3.32 mg/g.

The seven nurse plants successfully enriched with ^{15}N showed a mean enrichment ($\delta^{15}N_{After} - \delta^{15}N_{Before}$) of 355 ± 256‰ (Table 1). Thirteen out of the 14 facilitated species also showed an increase in their foliar $\delta^{15}N$ values after 15 d of the labeling of their nurse (mean = 1.08 ± 0.28‰; Table 2), meanwhile *E. eglandulosa*, showed slightly lower $\delta^{15}N$ values (change in $\delta^{15}N = -0.78$‰).

We found that ^{15}N transfer from the nurse to the facilitated plants increased with the phylogenetic distance between them (Fig. 2; posterior mean estimate of the slope = 0.006; probability of the slope being greater than 0 = 98.5%, Fig. 1).[15]N transfer followed a source–sink gradient, with increasing ^{15}N transfer toward those facilitated species generating steeper N_{tot} gradients (posterior mean estimate of the slope = 0.056; probability of the slope being greater than 0 = 99.3%, Fig. 1).

DISCUSSION

Classical explanations for the maintenance of facilitative interactions between distantly related species have suggested the avoidance of competitive exclusion through niche segregation (Webb et al. 2002, Pausas and Verdú 2010). We show that a phylogenetic signal in the source–sink N gradients among coexisting plant species correlates with an enhancement of inter-plant N transfer between distantly related species. This phylogenetic pattern in the positive interactions of nutrient sharing provides a new, non-exclusive, functional mechanism that may also contribute to explain coexistence of distantly related plants (Valiente-Banuet et al. 2006, Valiente-Banuet and Verdú 2007, 2013, Castillo et al. 2010).

Facilitative interactions initially allow plant coexistence by providing suitable regeneration niches for distantly related plant species, ultimately increasing the phylogenetic diversity of plant communities (Valiente-Banuet and Verdú 2007). Plant phylogenetic diversity increased by plant facilitation enhances soil microbial productivity and ecosystem functioning (Navarro-Cano et al. 2014). Our results shed light on a novel mechanism potentially enhancing facilitative interactions across distantly related species.

We found evidence of a statistically significant enhancement of inter-plant N transfer between distantly related species. *Echinopterys eglandulosa* showed no evidence of N transfer despite its large phylogenetic distance to the nurse *M. luisana*. However, this species had the highest foliar N_{tot} concentration of the sampled species, even higher than its nurse plant. Several processes can result in unexpected patterns like this. *Echinopterys eglandulosa* was associated with a nurse plant with relatively low foliar N concentration compared to other plants of *M. luisana* sampled (Patch D, Table 1). N-poor *M. luisana* individuals might tend to retain N instead of transferring it to other species, which would explain why this nurse plant, despite its high $\delta^{15}N$ enrichment, did not transfer much ^{15}N to its facilitated plants. This tendency might also explain the wide differences in the $\delta^{15}N$

enrichment among nurse plants, which did not show a clear correlation with the amount of ^{15}N transferred to their facilitated plants. However, despite the above mentioned ecological effects, there is evidence that foliar N concentration is evolutionarily conserved (Nakadai et al. 2014, Oguro and Sakai 2014, Werner et al. 2014), so that these exceptions to the general rule are unlikely to affect the generality of the reported phylogenetically conserved source–sink N gradient among species.

The increases in foliar $\delta^{15}N$ values found in facilitated plants after 15 d were relatively small (1.08‰ ± 0.28‰). However, it must be considered that firstly, the magnitude is high enough that it is unlikely to be driven by natural temporal variation in foliar $\delta^{15}N$ (Garten 1993), and secondly, that N transfer can be accumulated over time. On one hand, when ^{15}N transfer is relatively small, natural temporal fluctuations in foliar $\delta^{15}N$ values might blur the isotopic signal resultant from the transfer of the ^{15}N-enriched tracer. Thus, in order to minimize natural $\delta^{15}N$ variation in the focal facilitated species, we allowed a relatively short period of time for ^{15}N transfer to occur (15 d). The mean standard error of foliar $\delta^{15}N$ for the five species for which we could estimate intra-specific variation across individuals was 0.8, and the intra-individual temporal variation has been shown to be even smaller (Appendix S2). The magnitude of intra-individual fluctuations in foliar $\delta^{15}N$ for deciduous trees between spring and fall has been shown to be <0.3‰ (Garten 1993), and similar temporal fluctuations have also been reported for root $\delta^{15}N$ values (Sierra et al. 2007). Thus, considering that the increases in leaf $\delta^{15}N$ values reported here are above 0.3‰ for 7 of the 14 species, natural temporal variations of foliar $\delta^{15}N$ values are not likely to strongly affect the pattern shown here. On the other hand, the relatively short period of time allowed for ^{15}N transfer to occur might have reduced the recovery of ^{15}N in the facilitated species. However there is evidence that interplant N transfer can increase considerably when measured over longer periods (Sierra et al. 2007, Jalonen et al. 2009*a*, *b*). Taking this into consideration, the modest increase in leaf $\delta^{15}N$ values reported here might be ecologically relevant in the long term considering that N-transfer estimates can reach up to 30–40% of the total N in the receiver plants when measured over several months (Høgh-Jensen and Schjoerring 1997, Rasmussen et al. 2007). Finally, we consider it unlikely that the observed pattern is driven by interspecific differences in the natural rates of leaf ^{15}N accumulation. We showed previously in a simultaneous experiment similarly measuring ^{15}N transfer from nurse plants to their facilitated species (Montesinos-Navarro et al. 2016), that leaf $\delta^{15}N$ enrichment across facilitated plant species was significantly reduced when soil fungal abundance was reduced using a fungicide. This implies that the leaf $\delta^{15}N$ enrichment observed in facilitated plants was due to actual ^{15}N tracer transfer from the nurse plants, and not driven by interspecific differences in the rates of natural ^{15}N accumulation.

Two strands of knowledge together suggest that inter-plant nutrient transfer can be a widespread mechanism enhancing facilitation between distantly related plant species: (1) the ubiquity of nodulating legume species as high quality nurses and (2) a phylogenetically structured N source–sink gradient across the angiosperms. Firstly, N-fixing species, like legumes, are crucial for the establishment of many species in semiarid environments due to their ability to modify the nutritional, physical and chemical properties of the soil, thus diminishing abiotic stress for other species (Barnes and Archer 1996, Flores and Jurado 2003, Liphadzi and Reinhardt 2006, Navarro-Cano et al. 2014). Secondly, only the closely phylogenetically related nodulating plant species have the ability to use an extra source of N (i.e., atmospheric N_2) through symbiosis with N_2-fixing rhizobacteria (Werner et al. 2014), which enables the emergence of phylogenetically structured N source–sink gradients in multiple ecosystems. Finally, inter-plant transfers along source–sink gradients have also been reported for other nutrients such as phosphorus and carbon (Bethlenfalvay et al. 1991, Frey and Schüepp 1992, Simard et al. 1997, 2012) and resources such as water (Egerton-Warburton et al. 2007, Querejeta et al. 2012), thus raising the possibility that the phylogenetically driven mechanism reported here could also be at play for inter-plant transfers of these other limiting resources. However, most of the current knowledge about N transfer has been generated in agro-ecosystems and based on a legume as a donor (Chalk 1996, He et al. 2009, Chalk et al. 2014). Further research is required to assess the ecological implications of N transfer in facilitative interactions when non-legumes are the main nurse species in the community.

From an evolutionary perspective, an immediate question arising from our results is how N transfer from N-rich plants to their neighbors can be evolutionary stable. We propose different reasons below deserving further research. First, trade-offs between the ability to capture nutrients and store them can result in a poor capacity of N-rich plants to retain N. Some traits allow a more rapid acquisition of resources from the rhizosphere, such as total root length (Craine et al. 2005, Olde Venterink 2011), while others prevent nutrient losses and favor storage, such as long leaf lifespan, defenses against herbivores, and high nutrient-resorption efficiency (Harrington et al. 2001, Treseder and Vitousek 2001, Güsewell 2005*a*, *b*, Lambers et al. 2008, 2010, Olde Venterink 2011). Each strategy is favored under different nutrient-limiting environments, so while acquisitive traits are favored in N-limiting environments, storage traits are favored under P limitation (Olde Venterink 2011). This suggests that potential trade-offs between these two strategies might emerge in environments colimited by multiple nutrients. Second, N-rich nurse species could transfer N in exchange for other resources received from their facilitated plant species. Plant species differ in their functional traits, and this can provide different benefits to neighbor plants. For example, grasses have been suggested to be

sinks (receivers) of N, whereas legumes can act as P sinks (Johansen and Jensen 1996, Meding and Zasoski 2008), providing the possibility for a mutual interspecific benefit through nutrient exchange. Reciprocal benefits mediated by complementary resource acquisition patterns have been shown for two shrub species intimately interacting in arid environments of southeastern Spain (Tirado et al. 2015). *Maytenus senegalensis* benefits from the presence of *Whitania frutescens* by increasing its water potential, while the latter benefits from the former by increasing its leaf P status (Tirado et al. 2015). Although the specific mechanisms remain largely unexplored, there is evidence of a long-term mutualism between plant species in our study system, suggesting that plant-plant exchange of resources may be involved in this mutualism. Sortibrán et al. (2014) showed that the nurse species *M. luisana* produced more flowers and fruits when it had facilitated plants growing underneath its canopy than when growing alone. Furthermore, nurse fitness increased with the phylogenetic diversity of the plant neighborhood (Sortibrán et al. 2014), a benefit that disappeared when the mycorrhizal fungal network in the shared rhizosphere was experimentally disrupted (L. Sortibrán, *personal communication*). Most of the plant species in our system form symbiosis with arbuscular mycorrhizal fungi (Camargo-Ricalde et al. 2003), which establish common mycorrhizal networks that enhance nutrient transfer between plant species (He et al. 2003, Jalonen et al. 2009*b*). This supports previous evidence that mycorrhizal fungi play a key role in inter-plant nutrient exchange (He et al. 2009, Chalk et al. 2014), potentially shaping plant facilitation interactions. Whether plant–mycorrhizal relationships can also contribute to the observed phylogenetic pattern in the amount of inter-plant N transfer is still to be explored.

In conclusion, although more comprehensive measures of plant fitness will be necessary to assess long term community dynamics, we document a phylogenetic signature imprinted by a nutrient sharing mechanism in a natural plant community, providing a novel mechanism that may help to explain coexistence between distantly related species. While the traditional explanations of coexistence have been framed within the competition paradigm, the proposed complementary mechanism is based on positive interactions of nutrients sharing. Our results provide intriguing clues in the search for phylogenetic patterns in the inter-plant transfer of other limiting nutrients, and open new research avenues to deepen current understanding of the coexistence mechanisms that maintain the phylogenetic diversity of plant communities.

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SUPPORTING INFORMATION