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Research

Mycorrhizal symbiosis increases the benefits of plant facilitative interactions

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The diversity of pathways through which mycorrhizal fungi alter plant coexistence hinders the understanding of their effects on plant-plant interactions. The outcome of plant facilitative interactions can be indirectly affected by mycorrhizal symbiosis, ultimately shaping biodiversity patterns. We tested whether mycorrhizal symbiosis enhances plant facilitative interactions and whether its effect is consistent across different methodological approaches and biological scenarios. We conducted a meta-analysis of 215 cases (involving 21 nurse and 29 facilitated species), in which the performance of a facilitated plant species is measured in the presence or absence of mycorrhizal fungi. We show that mycorrhizal fungi significantly enhance plant facilitative interactions mainly through an increment in plant biomass (aboveground) and nutrient content, although their effects differ across biological contexts. In semiarid environments mycorrhizal symbiosis enhances plant facilitation, while its effect is non-significant in temperate ecosystems. In addition, arbuscular but not ecto-mycorrhizal (EMF) fungi significantly enhance plant facilitation, particularly increasing the P content of the plants more than EMF. Some knowledge gaps regarding the importance of this phenomenon have been detected in this meta-analysis. The effect of mycorrhizal symbiosis on plant facilitation has rarely been assessed in other ecosystems different from semiarid and temperate forests, and rarely considering other fungal benefits provided to plants besides nutrients. Finally, we are still far from understanding the effects of the whole fungal community on plant-plant interactions, and on plant species coexistence.

Keywords: biotic mechanisms, facilitation, meta-analysis, mycorrhizal symbiosis, plant community, plant-plant interactions.

Introduction

Assessing which processes can underlie the assembly of communities has been a general goal in ecology for decades (Clements 1916, Gleason 1926). In the case of plant communities, facilitative interactions can drive plant community assembly processes by expanding species' niche, promoting ecosystem functioning and maintaining biodiversity (Bruno et al. 2003, Brooker 2006, Valiente-Banuet and Verdú 2013, McIntire

and Fajardo 2014, Bulleri et al. 2016). In plant facilitative interactions, one plant species (facilitated) is benefited from growing associated to another (nurse species), without resulting in any damage for the latter (Callaway 2007). Different mechanisms underlie positive effects of nurse on facilitated plants, not only through a reduction of abiotic stress (i.e. by the enhancement of water and nutrients availability, wind protection or soil oxygenation) but also by ameliorating or increasing the effects of other organisms (i.e. herbivory reduction, increase of seed dispersal or pollination, or plant-soil microbe feedbacks) (Callaway 2007). Plants can depend on plant-soil feedbacks to mobilize, absorb and exchange essential nutrients, and in turn they can enhance the microbial activity and mycorrhizal networks underneath their canopy (Van Der Putten 2009, Rodríguez-Echeverría et al. 2016). Mycorrhizal fungi can alter individual plant performance, as plants receive nitrogen (N) and phosphorus (P) from mycorrhizal fungi in exchange for plant-photosynthesized carbon (Smith and Read 1997, Wang et al. 2017). However, plant species differ in their competitive ability and mycorrhizal dependency, what can also influence the outcome of interactions between plants (Lin et al. 2015, Koziol and Bever 2016).

The effects of mycorrhizal fungi on plant-plant interactions can be studied using different methods, both in the field and under controlled conditions. The presence of mycorrhizal fungi can be either enhanced through inoculation treatments, or reduced both physically, using meshes or digging trenches, and chemically with the application of fungicide to the soil (Ouahmane et al. 2006, Babikova et al. 2013, Zhang et al. 2014, Montesinos-Navarro et al. 2016a). Besides the inoculation of cultivated-fungal inoculums, mycorrhizal fungi can be indirectly enhanced by planting the facilitated species at increasing distances from the nurse (i.e. donor) plant, or applying soil collected underneath the nurse plant; either as a treatment itself or in combination with the aforementioned mycorrhizal alterations (Borchers and Perry 1990, Onguene and Kuyper 2002, Dickie et al. 2004, Teste and Simard 2008). This wide diversity of mycorrhizal treatments might have influenced the observed effects of mycorrhizal symbiosis on plant facilitative interactions. Therefore, a synthesis across field studies and greenhouse experiments using different treatments can assess whether the effect of mycorrhizal symbiosis on plant facilitation is consistent. If not, it might suggest that the methodology must be unified to obtain comparable results or strategically designed to address a given question from complementary approaches (Munafò and Smith 2018).

The magnitude of the effect of the mycorrhizal symbiosis on plant facilitation might depend on species responses to biotic interactions across ecosystems, functional differences between mycorrhizal types, or the specific components of plant performance considered. Plant facilitative interactions are widespread across contrasting ecosystems such as semiarid, alpine, and wetlands, but the proportion of studies reporting positive plant-plant interactions varies across ecosystems (Bonanomi et al. 2011). The relevance of mycorrhizal fungi for plant performance might be enhanced under stressful conditions, in soils with low water and nutrient availability, where the high potential of fungal hyphae to acquire resources can be crucial. Arbuscular mycorrhizal fungi (AMF) are associated with approximately 70% of the world's flowering plants, including most forbs and grasses, while a smaller proportion of plants (3%) is associated with ectomycorrhizal fungi (EMF), typically woody plants (Smith and Read 1997). EMF are known to decompose organic material benefiting from organic C and N (Read and Perez-Moreno 2003), although recent evidence support that AMF can also transfer N from organic compounds to their host plants (Leigh et al. 2009). This can result in differences between functional mycorrhizal types (i.e. AMF and EMF) regarding the proportion of resources provided to partner plants. Besides improving nutrients and water acquisition, both types of mycorrhizae can provide plant protection against pathogens (Azcón-Aguilar and Barea 1997, Borowicz 2001) and root connection through fungal mycelia, allowing the exchange of chemical signals that can induce defenses responses (Song et al. 2010, Babikova et al. 2013), allelochemicals (Barto et al. 2011), and nutrients among the connected plants (He et al. 2003, Selosse et al. 2006, Van der Heijden and Horton 2009). Furthermore, mycorrhizal richness and phylogenetic diversity can also relax plant-plant competition and promote plant coexistence (Van der Heijden et al. 1998, Maherali and Klironomos 2007, Wagg et al. 2011). However, only a few studies have focused on the influence of fungal richness and phylogenetic diversity on plant facilitative interactions (Van der Heijden et al. 1998, Wagg et al. 2011, Montesinos-Navarro et al. 2012, 2016b). Although our understanding of the role of mycorrhizal fungi on plant coexistence is expanding (Hart et al. 2003, Bever et al. 2010), we still ignore how the effect of mycorrhizal symbiosis on plant facilitative interactions varies across different biological scenarios.

Using a meta-analysis, we tested whether mycorrhizal symbiosis enhances plant facilitative interactions and explore potential sources of variation in the magnitude of this effect. Specifically, we formulate five questions regarding methodological (1–2) and biological aspects (3–5) potentially influencing the effect size: 1) Does mycorrhizal symbiosis promote plant facilitation under controlled (greenhouse, mesocosms) and field conditions? 2) Do the treatments applied to mycorrhizal fungi (e.g. inoculation, physical reduction with mesh treatments) influence plant facilitation? 3) Is mycorrhizal symbiosis more likely to enhance facilitative interactions in certain ecosystems? 4) Which plant traits are enhanced by plant facilitative interactions mediated by mycorrhizal fungi? 5) Do AMF and EMF have an effect on plant facilitation?

Material and methods

Following the methodological quality criteria for meta-analyses suggested by Koricheva and Gurevitch (2014) and Nakagawa et al. (2017) we a) report the full details of the

bibliographic search and the inclusion/exclusion criteria, b) weight the effect sizes by study precision, c) quantify heterogeneity in effect sizes, d) explore causes of heterogeneity, e) perform multiple analyses to assess the effects of moderators, f) test for publication bias, g) control for phylogeny and other sources of non-independence in the data, h) specify the software used in each case, i) provide a reference list of the studies included and the data set used for the meta-analysis, and j) summarize the current state (and lack) of knowledge on the topic approached.

Data set description

We searched for papers that evaluated the performance of facilitated species under two conditions: intact and altered mycorrhizal fungi. In February 2018, we compiled the data set by searching in web of science for the terms 'plant facilitation' and 'mycorrhiz*' from 1900 to 2016. This original search resulted in 183 references, and those abstracts from before 1991, which were non-available in web of science, were obtained directly from each journal site. After an initial assessment of the abstracts, 173 references were discarded, and 10 provided information that matched our selection criteria (see details below). The abstract of the references cited in those papers were also revised and 9 more studies that matched our selection criteria were included in the final data base following this procedure (Supplementary material Appendix Table A1, Fig. A1).

In all the papers used there was previous knowledge supporting that facilitative interactions can be relevant in the studied systems, and this information for each study is summarized in the Supplementary material Appendix 1 Table A2. Papers usually identify the nurse and facilitated plants on the basis of a) experimental information (e.g. experimental evidence of nutrients transfer of nutrients, herbivores' defense or mycorrhizal fungi provision from one species (nurse) to the other (facilitated); b) successional patterns (e.g. pioneer plants which are able to establish on the bare ground (nurse) and enhance the microenvironment allowing late-successional plant species (facilitated) to recruit); or c) study system information (e.g. plants are organized in vegetation patches resulting from facilitative interactions, which usually start with the establishment of a given plant species (nurse)). In the papers involving nutrient transfer, the donor plant was considered as the nurse, and the receiver as the facilitated. There is evidence to support that although a plant species can facilitate another species, the former can also get a benefit from the later thus resulting in a reciprocal benefit (Pugnaire et al. 1996, Castro et al. 2002, Sortibrán et al. 2014). In these cases, the two species can act both as nurse and facilitated. When previous knowledge supports that the two species can act as nurse and facilitated reciprocally, and the performance of both plants was provided, we consider each species as nurse and facilitated in two separate cases in our database.

We selected those papers that 1) identified a target facilitated species, 2) imposed a specific treatment that ensured that mycorrhizae abundance had been reduced or enhanced, 3) did not focus solely in agricultural systems, and 4) reported the mean and dispersion measure of the performance of the target species in contrasting mycorrhizal conditions. We excluded papers that reported the percentage of mycorrhizal colonization in the facilitated plant as the only measure of plant performance. This resulted in 215 cases from 19 studies, and we only considered for further analyses those methodological or biological aspects that were reported in not less than 10 cases in at least 3 papers. This criterion excluded alpine and wetland ecosystems, survival and herbivory from the plant performance measurements and root/shoot ratio records.

In order to assess the effect of mycorrhizal symbiosis on plant facilitative interactions we quantified the additional effect that the mycorrhizal symbiosis has on the facilitated plant. We considered studies that measured the performance of the facilitated plant species in different mycorrhizal treatments. In each case, the performance of the facilitated species was measured under two conditions, and we considered as 'control' (C) those conditions in which mycorrhizal abundance is supposed to be higher than in its 'treated' (T) pair.

Our meta-analysis assessed the effect of methodological (question 1–2) and biological (3–5) aspects on facilitative interactions mediated by mycorrhiza. We summarized the 5 questions approached by first referring to the name of the factor analyzed, and then to the comparisons between the levels tested within each factor:

(1) Experimental conditions: field and controlled conditions.
(2) Mycorrhizal treatment: inoculated, natural source, fungicide and physical reduction. Natural source treatment referred to the cases in which facilitated seedlings were transplanted at different distances of a mycorrhizal donor (for example a healthy nurse plant), and physical reduction included, for instance, the use of meshes or trenches to prevent mycorrhizal colonization. The inoculation treatments were considered 'control' and their paired unaltered environment 'treated', and the opposite for fungicide or physical reduction treatments.
(3) Ecosystem: temperate and semiarid. The classification into ecosystems was based on Gómez-Aparicio (2009) and Bonanomi et al. (2011). These authors defined broad sense categories, considering for example 'semiarid' as different types of water-limited ecosystems (arid, semiarid, Mediterranean) or 'temperate' as oak and coniferous forests, grasslands and shrublands growing in temperate climates.
(4) Performance measurement: biomass and nutrient content. For biomass and nutrient content, we distinguish between 4a) Plant part (above and belowground and total) and 4b) Nutrient (N and P).
(5) Mycorrhizal type: EMF and AMF.

We tested whether the improvement in N and P status differ between the mycorrhizal types. AMF and EMF might have a similar access to P but differ in their access to organic N, thus, differences in the amount of N provided to the plants

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by each mycorrhizal type can be expected (Aerts 2003). We also assessed whether the predominance of EMF in tree species of coniferous and oak temperate forest, where AMF were absent, can influence the effect size. In order to ensure that differences in the effect size across ecosystems are independent of mycorrhizal type, we tested for ecosystem effects considering only EMF.

When several performance measurements were provided by a single study, we considered them as separate cases, and when necessary, we statistically controlled for this dependency including study as a random factor (see data analyses section). All the data classifications used (e.g. mycorrhizal type, nurse and facilitated species) were obtained directly from the information reported in the primary studies.

Effect size

The compiled studies provided quantitative performance measurements of the facilitated species in two independent groups of plants (control and treated). Thus, we calculated the effect size using Hedges' g standardized mean difference (Hedges 1981), using large-sample approximation to compute the sampling variances. Most studies reported standard errors for control and treatment groups, as Hedges' g uses standard deviation, we have transformed standard error to standard deviation to estimate Hedges' values. This metric was positive (i.e. C > T) when the performance of the facilitated plant was enhanced by mycorrhizal fungi, and negative (i.e. C < T) when it was reduced. Hedges' g metric and the sampling variance (s^2) were calculated using the function 'escalc' in the package 'metafor' in R ver. 3.2.2. (Viechtbauer 2015).

Data analyses

We used a random-effects model to estimate the mean summary effect, the between-studies variance (τ^2), and the total variance. Statistical significance of the overall mean effect size was inferred fitting a Bayesian linear model, using the effect size as the response variable. We included the species of the facilitated plant, publication and the phylogenetic relationships among nurse plant species (based on a backbone mega-phylogeny for angiosperm (Zanne et al. 2014)) as random factors in order to account for potential non-independence of some cases in the data base. The phylogenetic relationships were generated with the R function 'S.PhyloMaker' (Qian and Jin 2016). This package uses the PhytoPhylo backbone mega-phylogeny, which is an updated and expanded version of the time-calibrated angiosperm species-level phylogeny (Zanne et al. 2014). The community phylogeny was produced by matching the family names of the plant species in our data base with those in the backbone phylogeny, using the R package 'ape' (Paradis et al. 2004). The overall mean estimate and its significance were assessed estimating the intercept and whether its 95% credibility interval excluded zero.

Following Nakagawa and Santos (2012), we calculated the percentage of 'true' variance of the effect size by calculating the total I^2 (the percentage of variance in effects that cannot be explained by measurement error). We calculated I^2 accounting for potential non-independence of the data. To do so, we based on the posterior mode estimates of the variance of the moderators fitted the Bayesian linear model. Following Higgins and Thompson (2002), we interpreted I^2 of 25, 50 and 75% as small, medium and large, respectively.

In order to assess potential publication bias after accounting for the structure of the data, we looked for asymmetry in a modified version of a funnel plot, using the residuals of the Bayesian linear model instead of the actual effect sizes (Nakagawa and Santos 2012). Then, we applied an Egger's regression to analytically test for asymmetry in the funnel plot. An intercept of the Egger's regression significantly different from zero suggests that there is evidence for publication bias. Finally, we corrected the estimated effect size for publication bias (i.e. the absence of certain publications due to the lack of statistical significance and/or direction of the results). We did so using a Bayesian 'fill in' Meta-analysis method (BALM) (Du et al. 2017), which accommodates several bias mechanisms and provides estimation for the effect size and its variance after accounting for them. This method has been suggested to outperform other methods such as trim and fill that fail to provide accurate parameters estimate for meta-analyses with heterogeneous effects (Peters et al. 2007, Stanley and Doucouliagos 2014). We used independent Bayesian linear models to assess the impact of putative factors on the effect size. We performed a Bayesian linear model for each factor described above (i.e. experimental conditions, mycorrhizal treatment, ecosystem, performance measurement and mycorrhizal type).

In each Bayesian linear model, we used a non-informative prior, with an expected mean value of mu = 0 and a (co)variance matrix of V = I × 1e + 10, where I is an identity matrix. The model settings were chosen to store 1000 MCMC (Markov chain Monte Carlo) iterations with an autocorrelation between successive stored iteration less than 0.1. This was usually reached using 62 500 iterations, thinned every 50 and discarding a burn-in period of 25% of the iterations. The significance of the models was corrected for multiple testing following the FDR method, using the 'p.adjust' function implemented in R, and the models were performed with the package 'MCMCglmm' (Hadfield 2010) implemented in R ver. 3.2.2. (R Core Team).

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.173r3j7> (Montesinos-Navarro et al. 2018).

Results

Our selection criterion resulted in 215 cases from 19 studies, covering 29 species facilitated by 21 nurse species (Supplementary material Appendix 1 Table A3). There is a

similar number of cases conducted in the field and under controlled conditions (Fig. 1). There are more records in semiarid than temperate ecosystems (Fig. 1), and the most reported performance measurement is above ground biomass (Fig. 1). The sample size is balanced for cases considering N and P content, and different types of mycorrhizal fungi (Fig. 1). Finally, all studies conducted in temperate ecosystems involve plant species associated to EMF (20 cases), and 70% of EMF studies focused on conspecific facilitative interactions (self-facilitation) (83 cases).

We found that the overall effect of mycorrhizae on the performance of the facilitated plants was significantly positive (mean effect size= 0.74; 95% credibility interval (CI): (0.13, 1.30)). We found that most of the variance in effect sizes was attributable to real differences among studies ($I^2 = 90\%$; $\tau^2 = 1.93$). There was a high level of unexplained variance once the random factors have been considered (Residual $I^2 = 32\%$). Small amounts of I^2 were partitioned into the random factors publication and phylogenetic relationships ($< 0.0001\%$), but the identity of the facilitated species explained a considerable amount of between-studies variation (58%).

Both visual and analytical approaches suggest the presence of publication bias. The funnel plot using the residuals of the overall Bayesian linear model suggests some asymmetry (Supplementary material Appendix 1 Fig. A2), and Eggers regression shows an estimate of the intercept significantly higher from zero (estimate $\pm$ SE = 0.96 $\pm$ 0.3, df = 123,

p-value = < 0.01). We used BALM to correct for publication bias, as this method has been suggested to provide accurate estimation of effect sizes for meta-analyses with heterogeneous effects (Du et al. 2017), like those reported in this meta-analysis. This method does not consider potential non-independence of the data, and therefore the correction should be compared with the overall estimate of the mean effect size obtained from the Bayesian linear model without any random factor (mean effect size= 0.97; 95% credibility interval (CI): (0.76, 1.23)). After applying BALM correction, the estimate of the overall effect size was still significant (0.82; 95% CI (0.60 to 1.08)).

The effect of phylogenetic relationships and publication explained up to 9% and 6% of the mean variance in any model, respectively (Supplementary material Appendix 1 Table A4). The effect of the random variable 'facilitated plant species' explained a similar mean amount of variance (36–46%) in all the models in which it was included, except for ecosystem (semiarid vs. temperate) in which the variance explained was lower (8%) (Supplementary material Appendix 1 Table A4). The estimates of effect size in each multilevel-model were also significant after correcting for publication bias (Supplementary material Appendix 1 Table A5).

There was a significant positive effect of mycorrhizae on plant facilitative interactions in field studies (Fig. 1), meanwhile under controlled conditions, a wide variation across experiments resulted in a non-significant effect of mycorrhizae (Fig. 1). The effect sizes of studies that used fungicide,
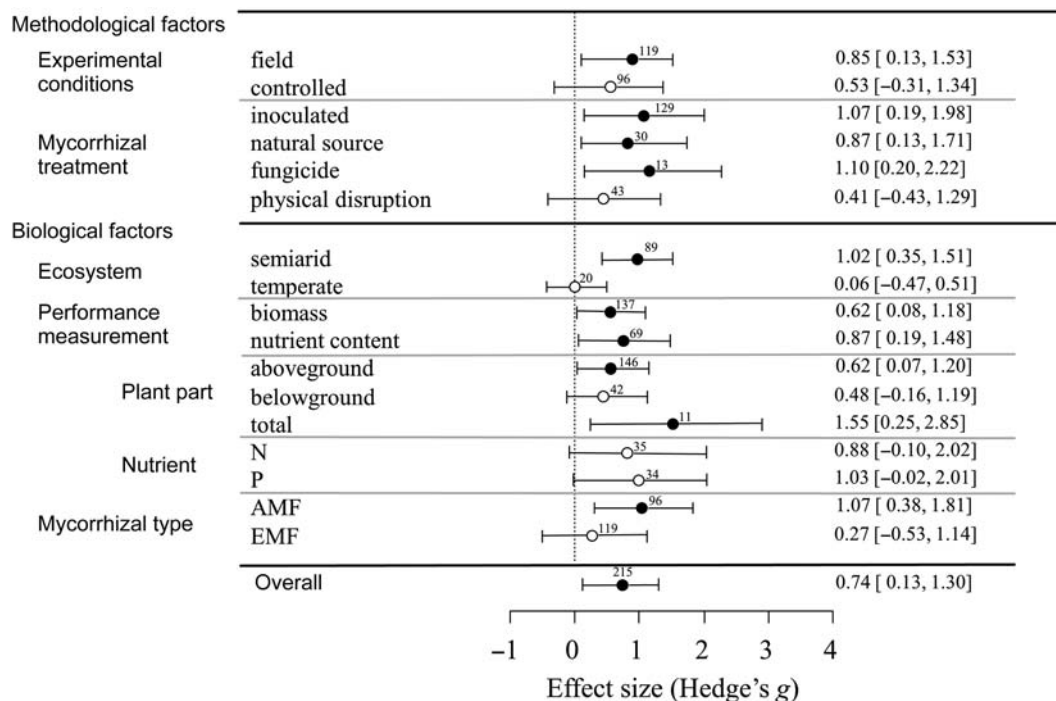

Figure 1. Effects of mycorrhizae on the performance of the facilitated plant under different methodological and biological scenarios. Mean and 95% credibility interval of the effect size estimate for each factor level and for the overall estimate. The number of cases is shown above the credibility intervals used to calculate them. Estimates were obtained independently for each factor. Black points indicate effect sizes estimates significantly different from 0. Mycorrhizal type refers to arbuscular mycorrhizal fungi (AMF) and ecto-mycorrhizal fungi (EMF).

inoculation or a natural source to alter mycorrhizae resulted in an enhancement of plant facilitation. However, the effect sizes of studies using physical disruption of the mycorrhizal fungi were non-significantly different from 0 (Fig. 1).

Mycorrhizal fungi significantly enhanced plant facilitative interactions in semiarid ecosystems but not in temperate ecosystems (Fig. 1). In addition, the overall effect of EMF on plant facilitative interactions was not significantly different from 0. When only EMF were considered, there was a significant positive effect on plant facilitative interaction in semiarid (mean effects size =1.08 CI = [0.18, 1.63]) but not in temperate ecosystems (0.06 [–0.36, 0.55]). The same comparison cannot be done for AMF as they are absent in the temperate studies compiled in our data set. Mycorrhizal fungi enhanced plant facilitative interactions by increasing plants biomass and nutrient status, improving both performance measurements with a similar magnitude. However, a significant positive effect was only observed when aboveground or the total plant biomass are considered, but the effect size was not statistically significant when only roots were considered (Fig. 1). Finally, we did not find a significant effect of mycorrhizal fungi on plant facilitation through an increment in nutrient content, as neither the plants N or P content were statistically significant (Fig. 1). However, our results showed a significant interaction between mycorrhizal type (AMF and EMF) and nutrient content (N and P) (Table 1). AMF enhanced facilitation through improving P content more than EMF, but the effect of AMF and EMF was similar regarding N content (Fig. 2).

Discussion

We found an overall enhancement of plant facilitative interactions when the plants coexist with mycorrhizal fungi. The traits conferring to the facilitated plant the ability to benefit from the mycorrhizal fungi of their nurses seem to be widespread in the phylogeny, given the low proportion of the variance explained by the phylogeny of facilitated species. Rather than the phylogenetic position of a species, it was its identity what explained a considerable amount of variation. In addition, the overall positive mean effect size was maintained in field conditions regardless of the performance measures used. However, the effect was not significant under controlled experimental conditions, what highlights the necessity to improve the realism of experimental studies (e.g., considering diversity of fungi, environmental heterogeneity, etc.). Alternatively, field studies could detect spurious facilitative
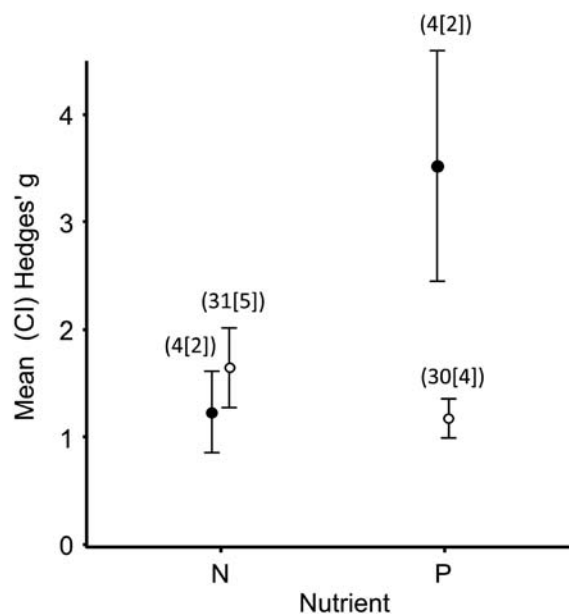

Figure 2. Interaction between the effect of different mycorrhizal types (arbuscular (AMF) vs ectomycorrhizal (EMF)) on plant nutrients contents (nitrogen (N) and phosphorous (P)). Mean and 95% credibility intervals of the effect size are presented for AMF (black circles) and EMF (white circles). Numbers indicate the number of cases [and studies] in each group.

interactions, but it seems unlikely that many uncontrolled factors in the field would result in the emergence of structured patterns. This meta-analysis is based on a relatively low number of studies (n = 19), and therefore the results should be taken with caution, although those studies compile a total of 215 cases, which is a considerably high sample size. Our results suggest the presence of publication bias, but once we corrected for it the effect size was still significantly positive.

Mycorrhizal fungi can enhance plant facilitation by connecting plants roots, allowing the transfer of resources between them (Francis et al. 1986, Smith and Read 1997, He et al. 2003, Wilson et al. 2006, Egerton-Warburton et al. 2008). This transfer tends to occur following source-sink gradients from a nurse plant with a higher N content to the facilitated (poor-N) plants (Montesinos-Navarro et al. 2017). However, our results show that the effect size is non-significant when mycorrhizal networks are physically disrupted with trenches or meshes, may be due to a non-effective reduction of fungal colonization with these methods. However, facilitation is promoted when mycorrhizae are reduced using chemical methods (fungicide), or added, either through inoculation or

Table 1. Bayesian linear model testing for the potential interaction between mycorrhizal type (arbuscular and ecto-mycorrhizal fungi) and nutrient type (nitrogen and phosphorus) on the effect size.

	Posterior mean estimate	Lower 95% credibility interval	Upper 95% credibility interval	pMCMC
Intercept	0.64	–0.66	1.94	0.34
Mycorrhizal type	0.19	–2.69	2.72	0.84
Nutrient type	–0.11	–0.57	0.38	0.63
Mycorrhizal × nutrient	2.02	0.80	3.20	< 0.001

through a mycorrhizae donor plant growing close to the facilitated plants. In 'natural source' treatment, other non-controlled factors besides fungal abundance, such as soil fertility or soil moisture can be enhanced under the nurse canopy. However, these other factors are unlikely to be relevant, as 'natural source' treatment does not show a remarkably different effect size compared to other experimental approaches (Fig. 1). Plant facilitative interactions can also be affected by the nutritional status of the plants involved, which can be influenced by mycorrhizal fungi. For example, plants with an improved nutritional status can provide more C to their symbionts obtaining a higher amount of N in reward (Kiers et al. 2011).

Our meta-analysis detected a strong bias towards some types of ecosystems. The effect of mycorrhizal fungi on plant facilitative interactions might be overlooked in some environments such as wetlands, alpine and tropical ecosystems, where plant facilitative interactions have been previously reported (Bonanomi et al. 2011). Our results show that mycorrhizal symbiosis is prone to enhance plant facilitative interactions in semiarid environments, while its effect is non-significantly different from zero in temperate environments. Arid and semiarid environments are characterized by having extreme temperatures, drought stress, and low nutrient availability, and under these physical stresses, water and nutrient mycorrhizal acquisition may become especially relevant (Bowles et al. 2018). The differences in the mycorrhizal effects on plant facilitation between semiarid and temperate ecosystems could be influenced by the predominance of tree species in temperate ecosystem, which are commonly associated to EMF instead of AMF mycorrhizal fungi. However, our results suggest that this explanation is unlikely as when only EMF are considered, they also promote facilitation in semiarid but not in temperate ecosystems.

Plants involved in facilitative interactions are favored by an increase in their biomass and nutrient content when mycorrhizal fungi are present, and this benefit is more evident in the aboveground plant parts. Optimal resource allocation can also influence the outcome of plant-soil feedbacks (Revillini et al. 2016), and thus optimal partitioning theory predicts that plants should allocate more biomass to the organ that will alleviate resource limitation (Bloom et al. 1985). Since fungal symbionts are more efficient capturing nutrients than plant roots (Smith and Read 1997), a lower allocation to root mass can be expected when the plant relies on mycorrhizal symbiosis for nutrient uptake. However, our results support this theory only partially, as root biomass did not present a negative signal, but a non-significant effect. Overall, facilitated plants do not benefit from a significant increment in N or P content in the presence of mycorrhizal fungi (Fig. 1). However, AMF and EMF differ in their effects on plant nutrients content, with AMF enhancing the plants P content more than EMF (Fig. 2). This is consistent with previous evidence showing that AMF can provide up to 80% of P and 25% of N host requirements (Marschner and Dell 1994). These differences can be attributed to the

fact that mycorrhizal fungi and plants may compete for N but not for P, as fungi have a higher requirement of N than plants (optimal C:N ratio is 33:1 for plant leaf tissue but only 10:1 for fungal hyphae (Allen et al. 2003)). Besides improving plant nutrient content, mycorrhizae can also provide a wide diversity of benefits to its symbiont plants. Our meta-analysis shows that most of the studies only consider increments in biomass and nutrient contents as potential benefits for facilitated plants. Only a few studies focus on how adult plants increase seedling survival through the transfer of nitrogen and carbon mediated by mycorrhizal fungi, the induction volatiles' production to avoid herbivore-insects or the enhancement of nutrient transfer among adult plants promoting long-lasting facilitative interactions (Teste et al. 2009, Babikova et al. 2013, Song et al. 2015, Montesinos-Navarro et al. 2016a). This suggests a gap of knowledge regarding these other potential ways by which mycorrhizal fungi benefit plant facilitative interactions.

The positive effect of AMF on plant facilitative interactions is significant, while this is not the case for EMF. A non-significant contribution of EMF to plant facilitation can be related to the fact that most of the studies focusing on EMF considered facilitation within a plant species (70% of the cases). In terrestrial ecosystems facilitation between conspecifics is much less frequent than between plants species, which can be due to a greater intraspecific competition (Dybzinski and Tilman 2009, Barabás et al. 2016). Alternatively, a lower frequency of facilitation between conspecifics has been attributed to plant-soil negative feedbacks (Bonanomi et al. 2010). Thus, negative plant-soil feedbacks in conspecific facilitative interactions may cancel out the beneficial effects of EMF on plant facilitation, potentially resulting in a non-significant effect of EMF on plant facilitative interactions.

This meta-analysis shows an overall enhancement of plant facilitative interactions when the plants coexist with mycorrhizal fungi. In addition, it reveals some gaps of knowledge worth exploring in future research. Firstly, the role of mycorrhizal fungi on plant facilitation could has been disregarded in alpine, wetland and tropical ecosystems where other mechanisms of facilitation (e.g. reduction of excessive temperature or solar radiation by shading, increase of temperature and protection from frost in cold climate, modification of soil nutrient fertility) have been previously described (Bonanomi et al. 2011). Secondly, only a few studies have considered alternative benefits that fungal symbionts can provide to their hosts. Some potentially disregarded benefits are mycorrhizal protection against pathogens or herbivores, attraction of pollinators, distribution of allelochemicals, or nutrient transfer from the nurse plant. Finally, we still lack a deep understanding of how community structure properties such as fungal richness or phylogenetic diversity can enhance facilitative interactions, despite the evidence that fungal richness or phylogenetic diversity can shape plant co-existence patterns (Maherali and Klironomos 2007, Montesinos-Navarro et al. 2012, 2016b). Thus, our results highlight the consistency of a positive effect of mycorrhizal symbiosis on

plant facilitative interactions, but further research is required to gain a deep mechanistic understanding of this ecological process.

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