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COMMUNITY ECOLOGY – ORIGINAL RESEARCH

Fungal phylogenetic diversity drives plant facilitation

Alicia Montesinos-Navarro[1,2] · **J. G. Segarra-Moragues**[1,3] · **A. Valiente-Banuet**[2,4] · **M. Verdú**[1]

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Abstract Plant–plant facilitation is a crucial ecological process, as many plant species (facilitated) require the presence of an established individual (nurse) to recruit. Some plant facilitative interactions disappear during the ontogenetic development of the facilitated plant but others persist, even when the two plants are adults. We test whether the persistence of plant facilitative interactions is explained by the phylogenetic diversity of mutualistic and non-mutualistic fungi that the nurse and the facilitated species add to the shared rhizosphere. We classify plant facilitative interactions as persistent and non-persistent interactions and quantify the phylogenetic diversity of mutualistic and non-mutualistic fungi added by the plant species to the shared rhizosphere. Our results show that the facilitated species add less phylogenetic diversity of non-mutualistic fungi when plant facilitative interactions persist than when they do not persist. However, persistent and non-persistent facilitative interactions did not differ in the phylogenetic diversity of mutualistic fungi added by the facilitated species to the shared rhizosphere. Finally, the fungal phylogenetic diversity added by the nurse to the shared rhizosphere did not differ between persistent and non-persistent interactions. This study suggests that considering the fungal associates of the plant species involved in facilitative interactions can shed light on the mechanisms of persistence for plant–plant interactions.

Keywords Community assembly · Aboveground–belowground · Phylogenetic structure · Plant facilitation · Fungal multifunctionality

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✉ Alicia Montesinos-Navarro
ali.montesinos@gmail.com

[1] Centro de Investigaciones sobre Desertificación (CIDE, CSIC-UV-GV), Carretera de Moncada-Náquera Km 4.5, 46113 Moncada, Valencia, Spain

[2] Departamento de Ecología de la Biodiversidad, Instituto de Ecología, Universidad Nacional Autónoma de México, A.P. 70-275, C.P. 04510 Mexico, D.F., Mexico

[3] Departamento de Biología Vegetal, Facultad de Ciencias Biológicas, Universitat de València, Avda. Dr. Moliner, 50, 46100 Burjassot, Valencia, Spain

[4] Centro de Ciencias de la Complejidad, Ciudad Universitaria, Universidad Nacional Autónoma de México, 04510 Mexico, D.F., Mexico

Introduction

The role of biotic interactions as drivers of community assembly has traditionally interested ecologists, who have provided multiple examples of how interacting guilds can reciprocally shape their species assemblages (Janzen 1970; Connell 1971; Packer and Clay 2000; Wolfe et al. 2005; Cahill et al. 2008; Waterman et al. 2011; Montesinos-Navarro et al. 2015). In the case of plant communities, plant species composition can influence the assembly of mutualistic and non-mutualistic partners (Thrall et al. 2007; Encinas-Viso et al. 2012), which in turn can influence plant–plant interactions through indirect effects (Janzen 1970; Connell 1971; Van der Putten et al. 2001; Montesinos-Navarro et al. 2012a). When interactions between mutualistic and non-mutualistic plant partners occur, the outcome of plant–plant interactions and their persistence may be difficult to predict.

Plant facilitation is a crucial process structuring community assemblages (Valiente-Banuet and Verdú 2008) and maintaining biodiversity (Valiente-Banuet et al. 2006; Valiente-Banuet and Verdú 2007), especially in semiarid environments. Plant facilitation is a positive plant–plant interaction in which a nurse species provides another facilitated species with a regeneration niche without incurring any disadvantage to the nurse (Callaway 2007), which sometimes benefits both plants (Sortibrán et al. 2014). Plant facilitative interactions show certain specificity, resulting in some plant–plant pairs prevailing over others (Verdú et al. 2010). Two processes can result in the avoidance or non-persistence of a plant facilitative interaction. On one hand, the microclimate provided by a nurse can match the recruitment requirements of a subset of facilitated species in the community, but not all of them (Verdú et al. 2010). On the other hand, the benefits of the association can shift to competition along the ontogeny of the facilitated plant (Valiente-Banuet and Verdú 2008; Armas and Pugnaire 2009; Incerti et al. 2013; Rolo et al. 2013). However, when the benefits of plant facilitative interactions persist over time, there will be spatial associations between the adult individuals of the nurse and facilitated species (Valiente-Banuet and Verdú 2008).

The strength of facilitative interactions increases between plant species which have distinct arbuscular mycorrhizal fungi, probably reflecting a diverse functionality in the rhizosphere (Montesinos-Navarro et al. 2012a). However, it is usually difficult to assess the functional profile of the fungal community because few fungal traits with ecological relevance are known for most fungal taxa. When there is little functional information, the use of phylogenetic diversity can be convenient as the evolutionary relatedness between taxa is a good proxy of functional trait similarity (Cadotte et al. 2008); phylogenetic relationships have been suggested to broadly reflect species functional diversity.

Fungal functional diversity influences plant communities. Mycorrhizal fungal richness and phylogenetic diversity have been shown to promote plant coexistence and increase plant biomass (Van der Heijden et al. 1998; Maherali and Klironomos 2007; Wagg et al. 2011). However, non-mutualistic fungi, even if they are not strictly considered as pathogens, may compete for space with mutualistic fungi in the plant root (Filion et al. 1999; Bodker et al. 2002; Roger et al. 2013; Thonar et al. 2014). In this sense, a higher functional (and phylogenetic) diversity of non-mutualistic fungi might reduce the available niche for mutualistic fungi, potentially reducing plant performance and coexistence. In this study we hypothesize that the persistence of plant facilitative interactions is influenced by the phylogenetic diversity of mutualistic and non-mutualistic fungi added by the facilitated and nurse species to the shared rhizosphere. We calculate the relative fungal phylogenetic diversity added by the nurse and the facilitated plant species in 60 pair-wise plant–plant interactions. We tested whether persistent and non-persistent interactions differ in the fungal phylogenetic diversity added by the nurse and the facilitated species. We expect that in persistent interactions, the nurse and facilitated species will add more mutualistic and less non-mutualistic diversity to the shared rhizosphere than in non-persistent interactions.

Materials and methods

Study site

This work was performed in a natural community in the semiarid valley of Zapotitlán, in the state of Puebla, Mexico (18°20N, 97°28W) (Valiente-Banuet and Verdú 2008; Verdú et al. 2010; Montesinos-Navarro et al. 2012b). The vegetation in this area is a xeric shrubland dominated by the columnar cactus *Neobuxbaumia tetetzo* (J.M. Coult.) Backeb, *Agave* spp. and different species belonging to the families Fabaceae, Malpighiaceae, Verbenaceae and Asteraceae.

Plant–plant facilitation

The interactions studied involved six nurse species [*Acacia constricta* Benth., *Caesalpinia melanadenia* (Rose) Standl., *Eysenhardtia polystachya* (Ortega) Sarg., *Mimosa luisana* Bragndegee, *Mascagnia seleriana* Loes., *Senna wislizeni* (A. Gray) H.S. Irwin & Barneby] and ten facilitated species [*Agave karwinskii* Zucc., *Caesalpinia melanadenia*, *Coryphantha pallida* Britton & Rose, *E. polystachya*, *Justicia mexicana* Rose, *Mammillaria mystax* Mart., *Mammillaria collina* J.A. Purpus, *N. tetetzo*, *Ruellia hirsutoglandulosa* (Oerst.) Hemsl., and *S. wislizeni*]. Some species can be facilitated by a nurse, but act also as a nurse of other species (Valiente-Banuet and Verdú 2013). Sixty potential interactions were considered, including also those in which the same plant species can act as a nurse and as a facilitated plant (auto-facilitation). Different individuals of each species were considered according to their relative abundance in the community (Table S4). There is accumulated evidence in this system to support that plant–plant associations are driven by facilitation (Valiente-Banuet and Verdú 2007, 2008; Verdú et al. 2010; Verdú and Valiente-Banuet 2011), including experimental evidence for some species in the community (Castillo et al. 2010). Plant–plant facilitation matrices from Valiente-Banuet and Verdú (2008) and Verdú et al. (2010) were used to characterize plant–plant interaction persistence. In these studies, the number of seedlings growing beneath the canopies of

other species and in the bare ground were counted along four transects of 1,000 m in three different sites. This information was used to determine whether seedlings of facilitated species were spatially associated with adults of nurse species. Then, Valiente-Banuet and Verdú (2008) and Verdú et al. (2010) tested whether these interactions persisted over time by recording, along the same transects, the associations between adult individuals of the facilitated and nurse species. Therefore, the interaction persistence was not assessed for each single association of two individuals over time, but instead as an integrative measure of species distribution patterns at different developmental stages.

In this paper, we compare the seedling and adult plant facilitation matrices from Valiente-Banuet and Verdú (2008) and Verdú et al. (2010) to define the persistence of these plant–plant facilitation interactions. Persistent interactions were considered those in which both the seedlings and adults of the facilitated species were associated with the nurse plant (i.e. adult individuals of the two species coexist). Non-persistent interactions were those in which only the seedlings and not the adults of the facilitated species were spatially associated with a nurse, or those in which a potential nurse (species that acted as a nurse for some species in the community) did not facilitate a plant species that cannot recruit in bare ground (i.e. requires facilitation of other nurses to establish).

Root sampling design

This study uses a subset of the root samples collected for Montesinos-Navarro et al. (2012b), in two areas of 50 × 10 m each, representing the relative abundance of each species. This subset results from selecting only the plant species for which there was available information about the persistence of their interactions with other plants in Valiente-Banuet and Verdú (2008) and Verdú et al. (2010), in total 71 individuals of 13 plant species (number of individuals per species in Appendix Table TS4). As explained in Montesinos-Navarro et al. (2012b), roots from adult plants were unearthed, and 50 mg of the youngest tips of the non-lignified root segments was dried in silica gel for further DNA extraction and molecular characterization of the fungal assemblages of Glomeraceae and Hypocreales (details below).

We chose the order Hypocreales because it is rich in fungal plant pathogens (Zhang et al. 2006; Hirooka et al. 2012), including some of the most economically important plant crop pathogens of the genus *Fusarium* (Michielse and Rep 2009; Schroers et al. 2009). Nonetheless, it also includes saprobes or facultative pathogens (Zhang et al. 2006; Hirooka et al. 2012). Although not all Hypocreales will damage plant roots, we assume that as fungi compete for space in the plant roots (e.g. Filion et al. 1999; Bodker et al. 2002; Roger et al. 2013; Thonar et al. 2014), the presence of mutualistic instead of non-mutualistic fungi in the roots could be beneficial for the plant. For simplicity, hereafter we refer to Hypocreales as non-mutualistic fungi and to Glomeraceae as mutualistic fungi.

Fungal community

Glomeraceae (mutualistic fungi)

Glomeraceae partial 18S (small sub-unit) and internal transcribed spacer (full ITS) were amplified through a nested polymerase chain reaction (PCR). The primary PCR was conducted in a 25-µl volume including 1× *Taq* buffer (Biotools, Madrid), 3 mM $MgCl_2$, 0.5 mM of each deoxynucleotide, 0.4 mg/ml BSA, 12.5 pmol each of NS5 (forward) and ITS4 (reverse) primers of White et al. (1990), 1 unit of *Taq* DNA polymerase and 1 µl of crude DNA extract. The PCR program consisted of an initial DNA melting step of 3 min at 95 °C followed by 30 cycles each of 30 s at 95 °C, 30 s at 51 °C for annealing and 2 min at 72 °C for extension. After a final extension step of 10 min at 72 °C, PCRs were kept at 4 °C. One microlitre of this PCR was used as a template for the nested PCR. The PCR cocktail was identical to that of the primary PCR except for the primer pair used, which included Forward/Reverse, Glom1310/ITS4i (Redecker 2000; Redecker et al. 2003), for the amplification of *Glomus* group A (Schüßler et al. 2001). The PCR program consisted of an initial DNA melting step of 3 min at 95 °C followed by 30 cycles each of 45 s at 95 °C, 50 s at 56 °C for annealing and 1.5 min at 72 °C for extension. After a final extension step of 10 min at 72 °C, PCRs were kept at 4 °C. PCR products were checked on 1 % agarose gels.

PCR products were checked for positive amplifications of the expected size on 1 % agarose gels and then cloned into pGEM-T easy vector (Promega) and transformed onto 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, isopropyl β-D-1-thiogalactopyranoside ampicillin, Luria broth agar plates. Positive colonies were screened with T7 and SP6 vector primers for inserts of appropriate size, then cultured for miniprep plasmid extraction (Roche) and sequenced with the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). Sequencing was performed by Macrogen, Seoul. Forward and reverse sequences were compared, assembled and corrected where necessary using SEQUENCHER (Gene Codes, MI), thus establishing the consensus sequence of each sample.

One thousand and fifty-eight sequences (Genbank numbers in Appendix Table TS1) gave high Basic Local Alignment Search Tool (BLAST) scores (average 1,574.97, range 953–1,954) to *Glomus* s.l. taxa. BLAST searches

identified nine taxa that gave high maximum identity scores (95.85, 86–98) to Genbank sequences. The vast majority of the sequences (91.49 % of the 1,058 sequences) were assigned to *Rhizophagus irregularis* (Blaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler followed by 5.20 % of the sequences to *G. indicum* Blaszk. However, for *R. irregularis* BLAST identity scores ranged from 89 to 98 %, which may indicate intraspecific variability, or alternatively, a poor taxonomic knowledge of this species complex, thus suggesting an underestimation of the real number of taxa by BLAST searches.

Hypocreales (non-mutualistic fungi)

A nested PCR protocol was used for the amplification of fungal rDNA ITS from sampled roots. The primary PCR cocktail and amplification program followed the same procedures as those described above for *Glomus* except for the PCR reaction volume which totalled 12.5 µl. One microlitre of this PCR was used as the template for the nested PCR. The PCR cocktail (25 µl total volume) included 1× *Taq* buffer (Biotools), 2 mM $MgCl_2$, 0.5 mM of each deoxynucleotide, 12.5 pmol each of ITS1F (forward) of Gardes and Bruns (1993) and NL4 (reverse) of O'Donnell (1992) primers, 1 unit of *Taq* DNA polymerase and 1 µl of primary PCR as template. The PCR program consisted of an initial DNA melting step of 3 min at 95 °C followed by 30 cycles each of 45 s at 95 °C, 50 s at 55 °C for annealing and 1.30 min at 72 °C for extension. After a final extension step of 10 min at 72 °C PCRs were kept at 4 °C.

PCR products were checked for positive amplifications, cloned and sequenced following the same protocols described above for *Glomus*.

One hundred and twenty-nine sequences (Genbank numbers in Appendix Table TS2) gave high BLAST scores (average 1,665.44, range 929–1,796) to taxa of Hypocreales. BLAST searches identified eight taxa that gave high maximum identity scores (97.87, 86–100) to Genbank sequences. More than half of these taxa (68.99 % of the 129 sequences) corresponded to taxa of *Fusarium*, mostly concentrated in two taxa, *Fusarium solani* (Mart.) Sacc. (37.20 %), and *Fusarium oxysporum* Schltdl. f. *melonis* W.C. Snyder & H.N. Hansen (26.35 %) which have been reported as important plant pathogens (Michielse and Rep 2009).

DNA sequence alignment and analysis

Independent sequence alignments were built for Glomeraceae and Hypocreales datasets. Sequences were aligned with CLUSTALW (Thompson et al. 1994) implemented in MEGA4 (Tamura et al. 2007) and corrected where necessary using BioEdit version 7.0.9 (Hall, available at http://www.mbio.ncsu.edu/BioEdit/bioedit.html). As sequences of Hypocreales encompassed fungal species of different genera and families, alignment of the ITS1 region showed many ambiguous positions, thus subsequent analyses of this group of fungi were based on 5.8S-ITS2 and partial 28S rDNA regions.

The Glomeraceae phylogenetic tree was built by taking into consideration an ITS sequence of *Paraglomus* (Genbank accession number FN555285) as an outgroup to root the phylogenetic tree, whereas that of Hypocreales was rooted with an ITS sequence of *Xylaria hypoxylon* (L.) Grev. (Genbank accession number EU715670). Phylogenetic analyses of the nuclear ribosomal ITS sequences were carried out in the Cyberinfrastructure for Phylogenetic Research web portal (Miller et al. 2010) using the probabilistic maximum likelihood (ML) method, as implemented in the RAxML blackbox with the default settings (Stamatakis 2006; Stamatakis et al. 2008). Only sequences found in plant species for which facilitation interaction information was available were considered for further analyses.

Although the fungal community was characterized in each plant individual, the persistence or non-persistence of each plant interaction was not assessed at the individual plant, but at the plant species level. Independent consideration of each plant species' fungal community will result in pseudo-replication, as the persistence of the interaction is assigned at the species level. Thus we focused on the plant species level, and for the analyses we pooled the sequences obtained in all the individuals of the same plant species (Fig. 1). This resulted in a homogenization of the variation due to factors such as spatial distribution, neighbourhood composition or developmental stages across plant species.

Characterization of the interactions

We characterized each plant–plant facilitation interaction based on the phylogenetic diversity of the fungal associates added by the facilitated and the nurse species. We used the phylogenetic trees of mutualistic and non-mutualistic fungi to obtain the phylogenetic distance between each possible pair of DNA sequences in each tree. We considered two sets of sequences (a) the sequences associated with each plant species (Fig. 1A, B), and (b) the combination of sequences associated with either of the two plant species involved in a facilitative interaction (i.e. plant–plant interaction) (Fig. 1C). For a given set of sequences, we calculated the mean of the phylogenetic distances between all possible pairs of DNA sequences within that set, hereafter called mean phylogenetic diversity (MPD), always considering Glomeraceae and Hypocreales separately. Thus, for each set, the MPD is the sum of all the phylogenetic distances between each pair of sequences divided by the number of sequences in each set,

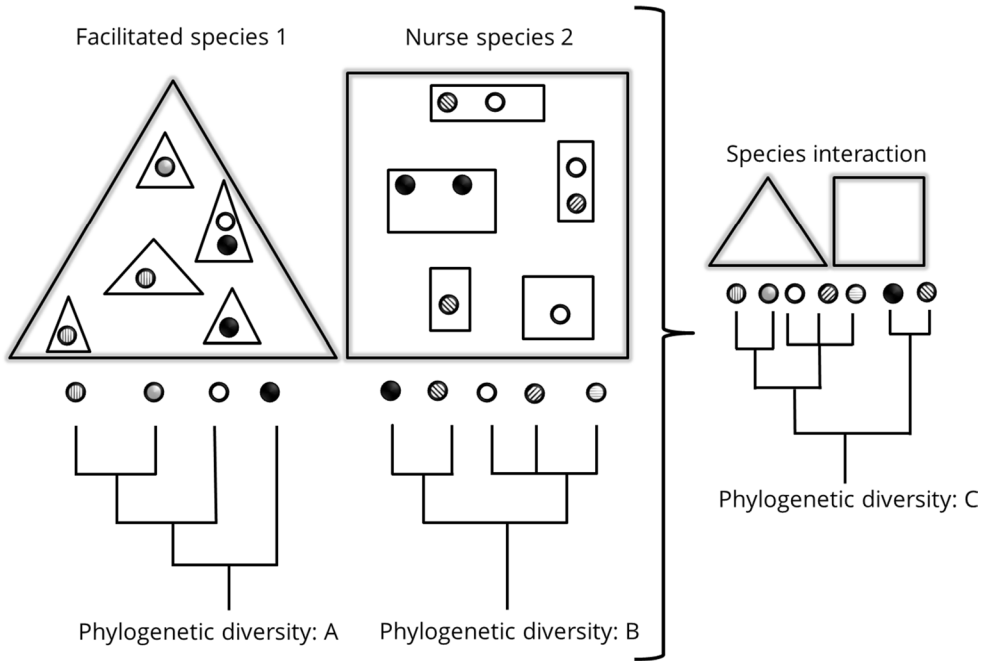

Fig. 1 Diagram representing the nature of plant–plant interactions considering mutualistic and non-mutualistic fungi independently. *Large triangles* represent a facilitated species and *large rectangles* a nurse plant species, and *small ones* within them plant individuals. *Differently patterned circles* represent different fungal DNA sequences harbored in each plant. The mean phylogenetic distance between every pair of different fungal DNA sequences is calculated for each plant species (*A*, *B*) and for the interaction (*C*) separately for mutualist and non-mutualistic fungi. The phylogenetic diversity that the facilitated species adds to the nurse species is the phylogenetic diversity of the interaction minus that of the nurse species (*C*–*B*). The phylogenetic diversity added by the nurse to the facilitated species is the phylogenetic diversity of the interaction minus that of the facilitated species (*C*–*A*)

and therefore it accounts for the differences in the number of sequences in different sets. Nevertheless, considering that plant species with more individuals sampled might have more DNA sequences, we checked that there was not a significant correlation between the number of individuals per plant species and the MPD, in either mutualistic ($r = 0.45$, $n = 13$, $t_{11} = 1.70$, $p > 0.05$) or non-mutualistic ($r = 0.50$, $n = 13$, $t_{11} = 1.93$, $p > 0.05$) fungi (for more details see the supplementary material, Methods appendix). We calculated the fungal phylogenetic diversity added by each plant species to the shared rhizosphere based on the MPD of fungi in each plant species, and the combined MPD in the two species involved in the plant–plant interaction. Thus, the contribution of the facilitated species to the shared rhizosphere was calculated as the difference between the MPD of Glomeraceae (or Hypocreales) of the plant–plant interaction minus the fungal MPD of the nurse (Fig. 1C−B). Similarly the contribution of the nurse was calculated as the difference between the fungal MPD of the plant–plant interaction minus the MPD of the facilitated species (Fig. 1C−A).

In this way, plant–plant interactions were characterized as the increment in MPD of mutualistic (MPD_{mut}) and non-mutualistic ($MPD_{non\text{-}mut}$) fungi added by the facilitated species (MutF and Non-mutF, respectively), and that added by the nurse species (MutN and Non-mutN) to the shared rhizosphere. Plant species or plant species pairs with a single DNA sequence of Glomeraceae or Hypocreales were assigned a MPD of zero for mutualistic and non-mutualistic fungi, respectively. MPD in all cases were calculated using the picante package for R (Stevens 2001), version 3.3.2.

Statistical analyses

We combined two type of analyses, at the individual plant level and at the plant–plant interaction level (pairs of species). The former allowed comparison of the fungal phylogenetic diversity of the nurse and the facilitated species, taking into account the number of individuals sampled per plant species. The latter was used to explore the factors explaining the plant–plant interaction persistence.

First, considering each individual plant, we compared MPD of mutualistic and non-mutualistic fungi associated with the nurse and the facilitated species. We used general linear mixed models with species and individuals as random factor. The MPD was log transformed to get a normal distribution of the residuals, although we presented the untransformed values of the MPD.

We explored the persistence of plant–plant interactions for 60 plant–plant interactions (six nurses and ten facilitated species; Appendix, Table TS3). We tested whether the interaction persistence was influenced by the MPD of mutualistic and non-mutualistic fungi added by the facilitated (Fig. 1C−B) and the nurse (Fig. 1C−A) species, respectively. To do this we performed two independent generalized mixed linear models. In both models we used a binomial distribution, as the dependent variable was the type of interaction (persistent vs. non-persistent). In the first model the explanatory variables were the MPD of mutualistic (MutF), non-mutualistic fungi (Non-mutF) and their interaction term (Non-mutF × MutF), added by the facilitated species (Fig. 1C−B). In the other model, the nurse species contribution (Fig. 1C−A) regarding the same variables (MutN, Non-mutN, Non-mutN × MutN) were used as explanatory variables. We also accounted for the effect of facilitated and nurse species as random factors in each model. Analyses were performed using the package lme4 (Bates et al. 2015) in for R version 3.2.2.

Table 1 Effects of the phylogenetic diversity of mutualistic (*MutF*) and non-mutualistic fungi (*Non-mutF*) added by the facilitated species and the nurse species on the persistence of the plant–plant facilitation interactions

Effects	Estimate	SE	*z*-value	*P*-value
Facilitated species contribution				
Intercept	0.226	0.668	0.339	0.74
Non-mutF	−23.884	10.984	−2.174	0.03
MutF	12.297	12.535	0.981	0.33
Non-mutF × MutF	12.768	175.106	0.073	0.94
Nurse species contribution				
Intercept	−0.143	0.953	−0.150	0.88
Non-mutN	−26.599	14.182	−1.876	0.06
MutN	−11.793	15.793	−0.747	0.45
Non-mutN × MutN	182.544	188.133	0.970	0.33

Generalized mixed linear models with a binomial distribution were used considering the interaction type (persistent vs. non-persistent) as dependent variable and accounting for the effect of the facilitated (or nurse) species as a random factor

Results

Nurse species had a greater MPD of mutualistic fungi (0.072 ± 0.014, $n = 25$; mean ± SE) than non-mutualistic fungi (0.054 ± 0.026, $n = 11$) (general linear mixed models, $t = -4.91$, $p < 0.01$). Facilitated species had a similar MPD of mutualistic (0.047 ± 0.007, $n = 50$) and non-mutualistic fungi (0.086 ± 0.021, $n = 26$) ($t = -0.79$, $p = 0.43$). When nurse and facilitated species were compared, the MPD of mutualistic fungi of the nurses was greater than that of the facilitated species ($t = 2.10$, $p = 0.04$), and the MPD of non-mutualistic fungi was similar between the nurse and the facilitated species ($t = -1.72$, $p = 0.12$, $n = 33$).

Regarding plant–plant interactions, the MPD of non-mutualistic fungi added by the facilitated species (Non-mutF) was significantly lower in persistent plant–plant interactions (0.04 ± 0.01, $n = 18$) than in non-persistent interactions (0.11 ± 0.02; $n = 42$; Table 1; Fig. 2a). However, the amount of MPD of mutualistic fungi added by the facilitated to the nurse did not significantly explain the persistence of facilitation (0.02 ± 0.01 in persistent vs. 0.02 ± 0.01 in non-persistent; Table 1). A similar pattern was observed regarding the nurse contribution. The MPD of non-mutualistic fungi added by the nurse was the only marginally significant factor explaining plant–plant interaction persistence (0.03 ± 0.01 in persistent and 0.07 ± 0.02 in non-persistent; Table 1b; Fig. 2b). The MPD of mutualistic fungi did not significantly explain the persistence of facilitation (0.02 ± 0.01 in persistent vs. 0.01 ± 0.01 in non-persistent; Table 1b).

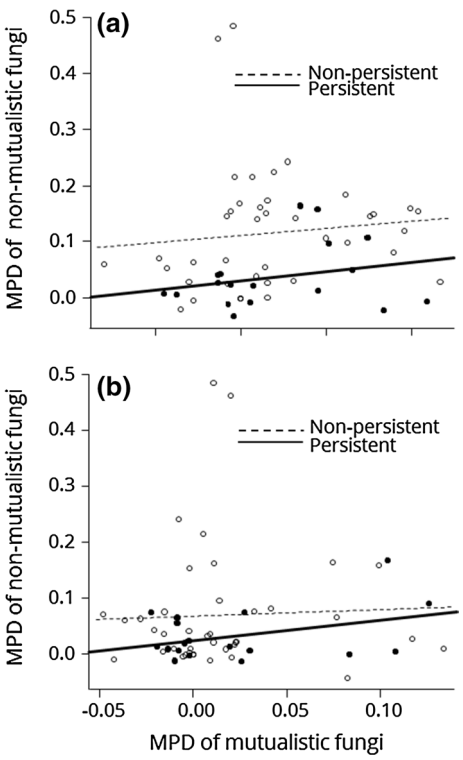

Fig. 2 The contribution of mutualistic and non-mutualistic fungi added by **a** the facilitated species [mean phylogenetic diversity (MPD) interaction – MPD facilitated] and **b** the nurse species (MPD interaction – MPD nurse) in persistent and non-persistent plant facilitative interactions. *Black circles* represent persistent plant–plant facilitation interactions, *white circles* represent non-persistent facilitation interactions

Discussion

We hypothesized that in persistent interactions, the nurse and facilitated species will add more mutualistic and less non-mutualistic diversity to the shared rhizosphere than in non-persistent interactions. Our results show that the fungal phylogenetic diversity of the plant species involved in plant–plant facilitation interactions differs between persistent and non-persistent interactions. In persistent plant–plant interactions the facilitated species adds less phylogenetic diversity of non-mutualistic fungi than in non-persistent interactions, and a similar pattern, although only marginally significant, is observed regarding the nurse species contribution. However, we do not find evidence to support that the MPD of mutualistic fungi, added by the nurse or the facilitated species, explains the persistence of the plant facilitative interactions. In addition, the role of the non-mutualistic fungi added by the facilitated species seems to have a greater influence on the persistence of plant facilitative interactions than the fungal MPD added by the nurse.

The sign of plant–plant interactions can shift from facilitation to competition due to temporal changes and during the ontogeny of the species involved (Miriti 2006; Schiffers and Tielbörger 2006; Armas and Pugnaire 2009; Soliveres et al. 2010). The fate of an interaction will therefore depend on whether the nurse and the facilitated species can coexist or whether they outcompete each other. Assessing different persistence scenarios in which either the nurse or the facilitated species outcompetes the other, can reveal the mechanisms underlying the causes of non-persistence of specific plant facilitative interactions. However, it is difficult to study the non-persistence of facilitative interactions because this requires following single interactions over a long time period in long-lived plants. However, it is easier to study the factors influencing the persistence of facilitative interactions. This study shows that the persistence of facilitative interactions can be influenced by minimizing the phylogenetic diversity of non-mutualistic fungi in the shared rhizosphere.

Plant coexistence can be influenced by the composition of a multi-species fungal assembly (Van der Heijden et al. 1998, 2003; Jansa et al. 2008) due to its effects on plant performance. Different mechanisms can lead to the enhancement of plant performance due to a high phylogenetic diversity of mutualistic fungi in the rhizosphere, such as complementary effects or selection effects (Maherali and Klironomos 2007). On one hand, a high phylogenetic diversity of mutualistic fungi can result in a high functional diversity due to complementary effects in terms of resource acquisition strategies or defence against pathogens (Newsham et al. 1995; Klironomos 2000; Jansa et al. 2008; Powell et al. 2009). At the same time, a high functional (i.e. phylogenetic) diversity of the non-mutualistic fungi assembly might reduce the niche available to mutualistic fungi coexisting in the root, due to competition for space in the plant root (Filion et al. 1999; Bodker et al. 2002; Roger et al. 2013; Thonar et al. 2014), although this has been largely unexplored. Consequently, a reduction of the functional diversity of non-mutualistic fungi could increase plant performance by reducing the competition for resources with mutualistic fungi within roots (Azcón-Aguilar and Barea 1996; Whipps 2004; Wehner et al. 2010). Most of the non-mutualistic fungi considered in this study belong to the genus *Fusarium*, and other Hypocreales which can also act as facultative pathogens, so a reduction of the phylogenetic diversity of non-mutualistic fungi may imply a lower diversity of infection strategies of potential fungal root pathogens. Alternatively, high phylogenetic diversity of fungi can benefit the plant due to selection effects (Grime 1998). A high phylogenetic diversity increases the probability of selecting particular fungal species that can enhance, in the case of mutualists, or reduce, in the case of pathogens, plant performance. For example, plant diversity and productivity depend more on the presence of certain mutualistic fungi species than on the fungal diversity per se (Vogelsang et al. 2006). In the case of non-mutualistic fungi, a low phylogenetic diversity can reduce the probability of selecting a particularly pathogenic fungi. This is consistent with our results showing that facilitation persists when the phylogenetic diversity of non-mutualistic fungi is minimized.

Our results indicate that specificity in plant facilitative interactions can be driven by the community of fungi added by the plant species to the shared rhizosphere (Valiente-Banuet and Verdú 2013). It has been shown that plants can regulate their associations with fungi through hormonal mechanisms. For example, the plant hormone jasmonate controls the production of chemical defence compounds that confer resistance to a wide spectrum of plant-associated organisms ranging from microbial pathogens to vertebrate herbivores (Campos et al. 2014). Jasmonate can also regulate mutualistic fungi colonization, and the fungal effects on floral traits (Kiers et al. 2010). This in turn can influence other biotic interactions in the community, potentially triggering cascade effects among multi-guild interactions influencing plant–plant facilitation. For example, it has been shown that AMF associates can influence plant–insect interactions (i.e. herbivory and pollination) through varying their foliar nutrient content (Barber et al. 2013), floral volatiles, flower colour and nectar composition (Cahill et al. 2008; Barber and Gorden 2015). In addition, plant–plant interactions can also be affected by indirect effects among multiple plant species in the neighbourhood (Castillo et al. 2010), potentially mediated by the multi-guild interactions occurring in the shared environment.

In summary, this study supports the hypothesis that the persistence of plant facilitative interactions can be partially

explained by an increase of the mutualistic and a reduction of the non-mutualistic fungal phylogenetic diversity that plants add to the shared rhizosphere. We provide an approach to consider multi-guild biotic interactions at the community level, which can shed light on the understanding of other ecological processes contributing to plant coexistence and the maintenance of diversity.

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Author contribution statement The manuscript was conceived with the contribution of all the authors: A. M. N., J. G. S.-M., A. V. B. and M. V.; A. V. B. collected the data; J. G. S.-M. performed the molecular analyses; all the authors discussed the analyses and results and provided comments during the manuscript preparation.

References

Armas C, Pugnaire FI (2009) Ontogenetic shifts in interactions of two dominant shrub species in a semi-arid coastal sand dune system. J Veg Sci 20:535–546

Azcón-Aguilar C, Barea JM (1996) Arbuscular mycorrhizas and biological control of soil-borne plant pathogens. An overview of the mechanisms involved. Mycorrhiza 6:457–464

Barber NA, Gorden NLS (2015) How do belowground organisms influence plant–pollinator interactions? J Plant Ecol 8:1–11

Barber NA, Kiers ET, Hazzard RV, Adler LS (2013) Context-dependency of arbuscular mycorrhizal fungi on plant–insect interactions in an agroecosystem. Front Plant Sci 4:338

Bates D, Maechler M, Bolker BM, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw. http://arxiv.org/abs/1406.5823

Bodker L, Kjoller R, Kristensen K, Rosendahl S (2002) Interactions between indigenous arbuscular mycorrhizal fungi and *Aphanomyces euteiches* in field-grown pea. Mycorrhiza 12:7–12

Cadotte MW, Cardinale BJ, Oakley TH (2008) Evolutionary history and the effect of biodiversity on plant productivity. Proc Natl Acad Sci USA 105:17012–17017

Cahill JF, Elle E, Smith GR, Shore BH (2008) Disruption of a belowground mutualism alters interactions between plants and their floral visitors. Ecology 8:1791–1801

Callaway RM (2007) Positive interactions and interdependence in plant communities. Springer

Campos ML, Kang JH, Howe GA (2014) Jasmonate-triggered plant immunity. J Chem Ecol 40:657–675

Castillo JP, Verdú M, Valiente-Banuet A (2010) Neighborhood phylodiversity affects plant performance. Ecology 91:3656–3663

Connell JH (1971) On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In: Den Boer PJ, Grandwell G (eds) Dynamics in populations. PUDOC, Wageningen, pp 298–312

Encinas-Viso F, Revilla T, Etienne RS (2012) Phenology drives mutualistic network structure and diversity. Ecol Lett 15:198–208

Filion M, St-Arnaud M, Fortin LA (1999) Direct interaction between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere microorganisms. New Phytol 141:525–533

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Grime JP (1998) Benefits of plant diversity to ecosystems: immediate, filter and founder effects. J Ecol 86:902–910

Hirooka Y, Rossman AY, Samuels GJ, Lechat C, Chaverri P (2012) A monograph of *Allantonectria*, *Nectria*, and *Pleonectria* (Nectriaceae, Hypocreales, Ascomycota) and their pycnidial, sporodochial, and synnematous anamorphs. Stud Mycol 71:1–210

Incerti G, Giordano D, Stinca A, Senatore M, Termolino P, Mazzoleni S, Bonanomi G (2013) Fire occurrence and tussock size modulate facilitation by *Ampelodesmos mauritanicus*. Acta Oecol 49:116–124

Jansa J, Smith FA, Smith SE (2008) Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi. New Phytol 177:779–789

Janzen DH (1970) Herbivores and the number of tree species in tropical forests. Am Nat 104:501–508

Kiers ET, Adler LS, Grman EL, van der Heijden MG (2010) Manipulating the jasmonate response: how do methyl jasmonate additions mediate characteristics of aboveground and belowground mutualisms? Funct Ecol 24:434–443

Klironomos JN (2000) Host-specificity and functional diversity among arbuscular mycorrhizal fungi. In: Bell CR, Brylinsky M, Johnson-Green P (eds) Microbial biosystems: new frontiers. Proceedings of the 8th international symposium on microbial ecology. Atlantic Canada Society for Microbial Ecology, Halifax, pp 845–851

Maherali H, Klironomos N (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. Science 316:1746–1748

Michielse CB, Rep M (2009) Pathogen profile update: *Fusarium oxysporum*. Mol Plant Pathol 10:311–324

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the gateway computing environments workshop (GCE) 14, pp 1–8

Miriti M (2006) Ontogenetic shift from facilitation to competition in a desert shrub. J Ecol 94:973–979

Montesinos-Navarro A, Segarra-Moragues JG, Valiente-Banuet A, Verdú M (2012a) Plant facilitation occurs between species differing in their associated arbuscular mycorrhizal fungi. New Phytol 196:835–844

Montesinos-Navarro A, Segarra-Moragues JG, Valiente-Banuet A, Verdú M (2012b) The network structure of plant–arbuscular mycorrhizal fungi. New Phytol 194:536–547

Montesinos-Navarro A, Segarra-Moragues JG, Valiente-Banuet A, Verdú M (2015) Evidence for phylogenetic correlation of plant–AMF assemblages? Ann Bot 115:171–177

Newsham KK, Fitter AH, Watkinson AR (1995) Multi-functionality and biodiversity in arbuscular mycorrhizas. Trends Ecol Evol 10:407–411

O'Donnell K (1992) Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete *Fusarium sambucinum* (*Gibberella pulicaris*). Curr Genet 22:213–220

Packer A, Clay K (2000) Soil pathogens and spatial patterns of seedling mortality in a temperate tree. Science 404:278–281

Powell JR, Parrent JL, Hart MM, Klironomos JN, Rillig MC, Maherali H (2009) Phylogenetic trait conservatism and the evolution of functional tradeoffs in arbuscular mycorrhizal fungi. Proc R Soc B Biol Sci 276:4237–4245

Redecker D (2000) Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots. Mycorrhiza 10:73–80

Redecker D, Hijri I, Wiemken A (2003) Molecular identification of arbuscular mycorrhizal fungi in roots: perspectives and problems. Folia Geobot 38:113–124

Roger A, Colard A, Angelard C, Sanders IR (2013) Relatedness among arbuscular mycorrhizal fungi drives plant growth and intraspecific fungal coexistence. Int Soc Microb Ecol (ISME) 7:2137–2146

Rolo V, Plieninger T, Moreno G (2013) Facilitation of holm oak recruitment through two contrasted shurbs species in Mediterranean grazed woodlands. J Veg Sci 24:344–355

Schiffers K, Tielbörger K (2006) Ontogenetic shifts in interactions among annual plants. J Ecol 94:336–341

Schroers HJ, O'Donnell K, Lamprecht SC, Kammeyer PL, Johnson S, Sutton DA, Rinaldi MG, Geiser DM, Summerbell RC (2009) Taxonomy and phylogeny of the *Fusarium dimerum* species group. Mycologia 101:44–70

Schüßler A, Gehrig H, Schwarzott D, Walker C (2001) Analysis of partial Glomales SSU rRNA gene sequences: implications for primer design and phylogeny. Mycol Res 105:5–15

Soliveres S, DeSoto L, Maestre FT, Olano JM (2010) Spatio-temporal heterogeneity in abiotic factors modulate multiple ontogenetic shifts between competition and facilitation. Perspect Plant Ecol Evol Syst 12:227–234

Sortibrán L, Verdú M, Valiente-Banuet A (2014) Nurses experience reciprocal fitness benefits from their distantly related facilitated plants. Perspect Plant Ecol Evol Syst 16:228–235

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688

Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML Web servers. Syst Biol 57:758–771

Stevens PF (2001) Angiosperm phylogeny website. Version 6, May 2005. http://www.mobot.org/MOBOT/research/APweb/

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Thonar C, Frossard E, Smilauer P, Jansa J (2014) Competition and facilitation in synthetic communities of arbuscular mycorrhizal fungi. Mol Ecol 23:733–746

Thrall PH, Hochberg ME, Burdon JJ, Bever JD (2007) Coevolution of symbiotic mutualists and parasites in a community context. Trends Ecol Evol 22:120–126

Valiente-Banuet A, Verdú M (2007) Facilitation can increase the phylogenetic diversity of plant communities. Ecol Lett 10:1029–1036

Valiente-Banuet A, Verdú M (2008) Temporal shifts from facilitation to competition occur between closely related taxa. J Ecol 96:489–494

Valiente-Banuet A, Verdú M (2013) Plant facilitation and phylogenetics. Annu Rev Ecol Evol Syst 44:347–366

Valiente-Banuet A, Vital A, Verdú M, Callaway R (2006) Modern Quaternary plant lineages promote diversity through facilitation of ancient Tertiary lineages. Proc Natl Acad Sci USA 103:16812–16817

Van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72

Van der Heijden MGA, Wiemken A, Sanders IR (2003) Different arbuscular mycorrhizal fungi alter coexistence and resource distribution between co-occurring plant. New Phytol 157:569–578

Van der Putten WH, Vet LEM, Wackers FL (2001) Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. Trends Ecol Evol 16:547–554

Verdú M, Valiente-Banuet A (2011) The relative contribution of abundance and phylogeny to the structure of plant facilitation networks. Oikos 120:1351–1356

Verdú M, Jordano P, Valiente-Banuet A (2010) The phylogenetic structure of plant facilitation networks changes with competition. J Ecol 98:1454–1461

Vogelsang KM, Reynolds HL, Bever JD (2006) Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. New Phytol 172:554–562

Wagg C, Jansa J, Stadler M, Schmid B, Van der Heijden MGA (2011) Mycorrhizal fungal identity and diversity relaxes plant–plant competition. Ecology 92:1303–1313

Waterman RJ, Bidartondo MI, Stofberg J, Combs JK, Gebauer G, Savolainen V, Barraclough TG, Pauw A (2011) The effects of above- and belowground mutualisms on orchid speciation and coexistence. Am Nat 177:E54–E68

Wehner J, Antunes PM, Powell JR, Mazukatow J, Rilling MC (2010) Plant pathogen protection by arbuscular mycorrhizas: a role for fungal diversity? Pedobiologia 53:197–201

Whipps JM (2004) Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Can J Bot 82:1198–1227

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and amplifications. Academic Press, New York, pp 315–322

Wolfe BE, Husband BC, Klironomos JN (2005) Effects of belowground mutualism on an aboveground mutualism. Ecol Lett 8:218–223

Zhang N, Castlebury LA, Miller AN, Huhndorf SM, Schoch CL, Seifert KA, Rossman AY, Rogers JD, Kohlmeyer J, Volkmann-Kohlmeyer B, Sung GH (2006) An overview of the systematics of the Sordariomycetes based on a four-gene phylogeny. Mycologia 98:1076–1087