Received: 22 February 2024 | Accepted: 19 November 2024

DOI: 10.1111/1365-2435.14723

Functional Ecology

RESEARCH ARTICLE

Nutrient exchange within common mycorrhizal networks is altered in a multispecies environment

Veronika Řezáčová[1] | Joanna Weremijewicz[2] | Tereza Michalová[3]

[1]Crop Research Institute, Prague 6, Czech Republic

[2]Department of Biology, North Central College, Naperville, Illinois, USA

[3]Institute of Microbiology of the Czech Academy of Sciences, Prague 4, Czech Republic

Correspondence
Veronika Řezáčová
Email: rezacova@vurv.cz

Funding information
National Science Foundation Faculty Early Career Development Program (CAREER), Grant/Award Number: #2145142; Ministry of Agriculture of the Czech Republic, Grant/Award Number: MZE-RO0423; Czech Science Foundation, Grant/Award Number: 18-01486S

Handling Editor: Sandra Varga

Abstract

1. Although it is known that arbuscular common mycorrhizal networks (CMNs) mediate below-ground interactions between one or two species, little is understood about their role in mediating interactions among multiple, co-occurring plant species.
2. We investigated the CMN-mediated interactions among two Central European species, *Inula conyzae* and *Crepis biennis* within pots and the impact of a third plant, an invasive *Echinops sphaerocephalus*, on these relationships. We examined changes in C-to-P exchange within a CMN formed by *Funneliformis mosseae* sourced from Central Europe by tracking plant C cost with ^{13}C signatures of 16:1ω5 and P acquisition to hosts with ^{33}P only accessible to CMNs.
3. When only native plants were present, the C cost was consistent for both species, despite CMNs favouring *C. biennis* with P uptake. In the presence of *E. sphaerocephalus*, CMNs also favoured *C. biennis* with P, but while *C. biennis* and *E. sphaerocephalus* provisioned similarly large portions of ^{13}C, *I. conyzae* provided less. Mycorrhizal P acquisition, therefore, was the costliest for *E. sphaerocephalus*, which likely mitigated some *I. conyzae*'s C cost even though both received a low proportion of ^{33}P from CMNs.
4. *Echinops sphaerocephalus* altered mineral nutrient and C exchange proportions between native plants and their CMN, suggesting that this species alters below-ground plant interactions and that not only specific characteristic of plant host and fungal partner but also the wider plant community mediates resource exchanges between CMNs and individual plants.

KEYWORDS
^{13}C labelling, 16:1ω5, arbuscular mycorrhiza, below-ground interactions, interspecific plant competition, invasive plant species, mycorrhizal carbon cost, symbiotic ^{33}P benefits

1 | INTRODUCTION

The majority of terrestrial plants form associations with arbuscular mycorrhizal (AM) fungi (Glomeromycotina; Spatafora et al., 2016), which affect plant interactions (Řezáčová, Řezáč, Gryndler, et al., 2021; Řezáčová, Řezáč, Líblová, et al., 2021; Smith & Read, 2008; Štajerová et al., 2009; van der Heijden et al., 2015; Wipf et al., 2019) and ultimately influence plant community

composition (Awaydul et al., 2019; Callaway et al., 2001; Workman & Cruzan, 2016).

These mycorrhizal relationships are based on reciprocal rewards: AM fungi supply host plants with essential mineral nutrients, primarily phosphorus (Lekberg et al., 2010), in exchange for photosynthetically fixed C from plants (Bago et al., 2000; Pfeffer et al., 1999). As obligate symbionts (Lanfranco et al., 2016), AM fungi usually consume up to 10% of their host plant's C budget (Řezáčová, Konvalinková, et al., 2017). Conversely, in P-limited soil conditions, AM fungi can be the primary supplier of P for host plants (Bennett et al., 2017; Liao et al., 2018). These benefits are dynamic and can be influenced by environmental abiotic (Řezáčová, Zemková, et al., 2018) factors, host plant dependence on mycorrhizal associations (Khalil et al., 1994) and the identity of symbiotic partners (Řezáčová et al., 2020; Řezáčová, Slavíková, et al., 2018).

Due to the low host specificity of AM fungi, the hyphae of the same fungal genotype usually interconnect neighbouring plant roots in nature, regardless of plant species, in a 'common mycorrhizal network' (CMN; Muller, 2021; Selosse et al., 2006). CMNs can mediate plant interactions by distributing nutrients among interconnected individuals, which can ultimately support the weaker competitor or intensify competition among neighbouring plants (Merrild et al., 2013; Montesinos-Navarro et al., 2016; Walder & van der Heijden, 2015; Weremijewicz et al., 2016, 2018; Weremijewicz & Janos, 2013; Workman & Cruzan, 2016). Resource partitioning by CMNs among plants may be influenced by the C investment of host plants; the shared fungal partner generally allocates more mineral nutrients to plants that provide more C than those that provide less C (Hammer et al., 2011; Kiers et al., 2011; Lekberg et al., 2010). However, this biological market theory of 'reciprocal rewards' (Kiers et al., 2011) has been developed through carefully controlled in vitro experiments and may not always hold true in natural communities where many plant species with different mycorrhizal dependencies and relationships to various fungal genotypes coexist. Thus, while the concept of reciprocal rewards suggests a preference for more beneficial partners in terms of nutrient exchange, many studies have focused on interactions involving one or two species, potentially overlooking more complex dynamics in natural settings.

In glasshouse experiments, resource allocation by CMNs has been found to depend on a variety of factors such as the nutrient sink strength of the plants involved (Walder & van der Heijden, 2015; Weremijewicz et al., 2016) and variations in the plants' ability to profit from AM fungi (Johnson & Graham, 2013; Walder & van der Heijden, 2015). CMNs may favour more mycorrhiza-dependent plant species over less-dependent ones (Weremijewicz et al., 2018) or an invasive host plant over a native one (Awaydul et al., 2019). Although C_3 plants are often considered less dependent on AM fungi for mineral nutrient uptake than C_4 plants, results from glasshouse studies on CMN-mediated interactions among C_3 and C_4 plants have conflicting results. For example, it has been demonstrated that a C_3 plant can benefit significantly from a CMN without contributing much C to the fungus, while the interconnected C_4 plant may allocate a substantial amount of C but receive only a limited amount of mineral nutrient in return (Walder et al., 2012; Walder & van der Heijden, 2015). In contrast, the C_3 plant within Faghihinia and Jansa's (2022) study allocated more C to a CMN than the C_4 plant and ultimately received more P in return. Řezáčová, Zemková, et al. (2018), however, did not find differences in C investment to CMNs by C_3 and C_4 plants, and this relationship did not change with elevated temperatures. Similar to C_3 plants, weedy and invasive plant species are typically facultatively mycorrhizal (Lin et al., 2015; Richardson et al., 2000; Vogelsang & Bever, 2009), but there are no clear patterns in C for mineral nutrient exchange between hosts and fungi. Based on the results of Awaydul et al. (2019) and Weremijewicz et al. (2018) with invasive versus native plants and more versus less mycorrhiza-dependent plants, respectively, it can be assumed that the invasive plants will be capable of acquiring nutrients from shared CMNs differently from non-invasive plants, either more successfully or less successfully, depending on their AM fungal dependence. CMNs have been shown to preferentially transfer ^{15}N and P to an invasive plant over a native one when they receive more C from invasive species in return (Awaydul et al., 2019, 2023). Similarly, in Řezáčová et al. (2020), CMNs benefited an invasive species with P over native species. On the contrary, Řezáčová et al. (2022) found that CMNs either had no effect on the size of the P fraction acquired by invasive and native plants or benefited the native plant with P uptake over the invasive plant.

Despite ongoing research into the C for P exchange and the factors influencing this exchange, the consequences of CMNs for plant interactions among different species, especially those differing in their origins and spread rates, remain incompletely understood. A critical gap in current research is the examination of CMNs involving multiple plant species to elucidate how these networks influence nutrient exchanges and competitive dynamics. Further research involving multiple mycorrhizal plant species within shared CMNs (Faghihinia & Jansa, 2022) is warranted, with a focus on C-for-P exchange, rather than just an assessment of growth benefits.

The primary objective of this study was to investigate the C for P exchange between plants and CMNs in a multispecies plant community and to evaluate if extension of the plant community by one plant changes the proportions of exchanged C for P between plants and CMNs. The research was conducted on a model plant community composed of plant species co-occurring in Central European plant communities belonging to one family—Asteraceae. Specifically, we studied the effect of an invasive *Echinops sphaerocephalus* L. on the CMN-mediated plant interactions among two native species to Central European plant communities, *Inula conyzae* (Griesselich) Meikle and *Crepis biennis* L. We examined changes to the dynamics among *I. conyzae* and *C. biennis* and their interconnecting CMN by investigating C provisioning to the CMN and P exchange from the CMN when *E. sphaerocephalus* was present or not. We hypothesized that CMNs play a significant role in below-ground competition among mycorrhizal plants and that connecting another plant to the

CMN would affect C-to-P exchange within CMN interconnecting the two native species, potentially influencing the competitive dynamics of the native plants.

2 | MATERIALS AND METHODS

2.1 | Experiment design

In a glasshouse, we set out to model a Central European plant community by growing two native, perennial herbs *I. conyzae* and *C. biennis* in the presence or absence of invasive *E. sphaerocephalus* (Figure 1). Pots were either inoculated with a mycorrhizal fungus, *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schuessler 2010, which allowed the establishment of a CMN within pots, or not. We examined C-to-P exchange within CMNs using ^{13}C labelling of each individual species within pots and ^{33}P isotopic labelling of substrate within a root-free compartment (RFC). There were five replicates for each treatment, for a total of 70 pots.

2.2 | Plants and growth conditions

The plant species used in this study were chosen because they belong to the Asteraceae family, coexist in Central Europe and establish relationships with AM fungi. *E. sphaerocephalus* is native to Asia and southern and eastern Europe, and its new distribution range includes nearly all of central Europe and North America. *E. sphaerocephalus* is commonly invasive here, although it's spread into plant communities is not particularly dramatic; it spreads, but rather over shorter distances. *I. conyzae* and *C. biennis* are both native to Central Europe. However, they differ in their ability to spread, with *C. biennis* spreading easily, even over longer distances, whereas *I. conyzae* is more confined to natural habitats and does not spread (www.pladias.cz).

Seeds were collected from natural habitats and sowed directly into pots at the end of January (1 seed of *E. sphaerocephalus*, 3 seeds of *C. biennis* based on the previous germination tests) or pregerminated on filter paper in Petri dishes (*I. conyzae*) with subsequent transplanting of always one seedling into a pot. The plants were cultivated in a glasshouse from January to May 2019. The glasshouse maintained average day and night temperatures of 24 and 20°C, respectively. The light intensity level corresponded to about 40 klux sunlight (photosynthetic photon flux density being approximately 690 $\mu mol\, m^{-2}\, s^{-1}$). Day length was extended to 12 h using supplemental lighting (mercury discharge lamps providing a minimum photosynthetic flux density of 200 $\mu mol\, m^{-2}\, s^{-1}$ at plant level). The plants were watered daily as needed, one or two times a day, with each dose receiving approximately 100 mL of water. After 3 and 4 weeks, excess seedlings were removed, leaving one plant of each species in each pot. Five weeks after sowing seeds, each pot was provided with a weekly dose of 105 mL of the Long Ashton mineral nutrient solution (Hewitt, 1966), with the P concentration reduced to 20% of the original recipe (following Řezáčová, Slavíková, et al., 2017).

2.3 | Cultivation pots and substrate

Plants were cultivated in 4-litre pots (Figure 2), which were lined with a nylon mesh (with 1.2 mm opening) at the bottom, sterilized with 96% ethanol and then filled with a potting substrate. The substrate was composed of a well-mixed blend (volume-based) of 10% γ-irradiated (>25 kGy) field Central European soil, 45% autoclaved zeolite MPZ 1–25 from Zeopol (www.zeolity.cz, grain size 1–2.5 mm) and 45% autoclaved quartz sand (grain size <3 mm; for physicochemical properties, please see Řezáčová,

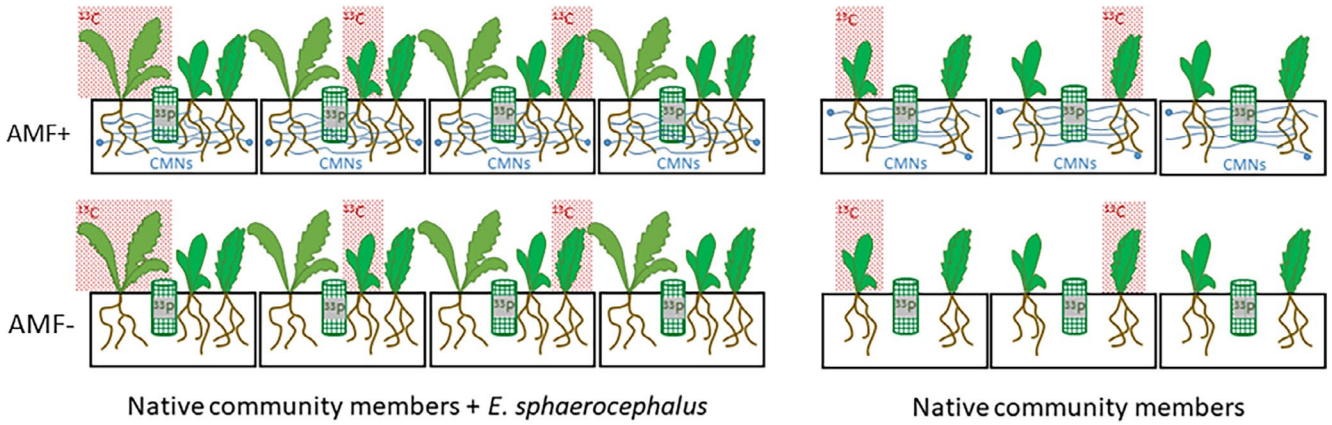

FIGURE 1 Diagram of the experiment design in which native plants *Inula conyzae* and *Crepis biennis* grown with or without the presence of *Echinops sphaerocephalus*, with (AMF+) or without (AMF–) arbuscular mycorrhizal fungi. Each individual species was exposed to $^{13}CO_2$ labelling (^{13}C, red frame) within the treatments. ^{33}P was added to a root-free compartment (green cylinder) in the centre of all pots that was only accessible to hyphae of *Funneliformis mosseae* (blue lines).

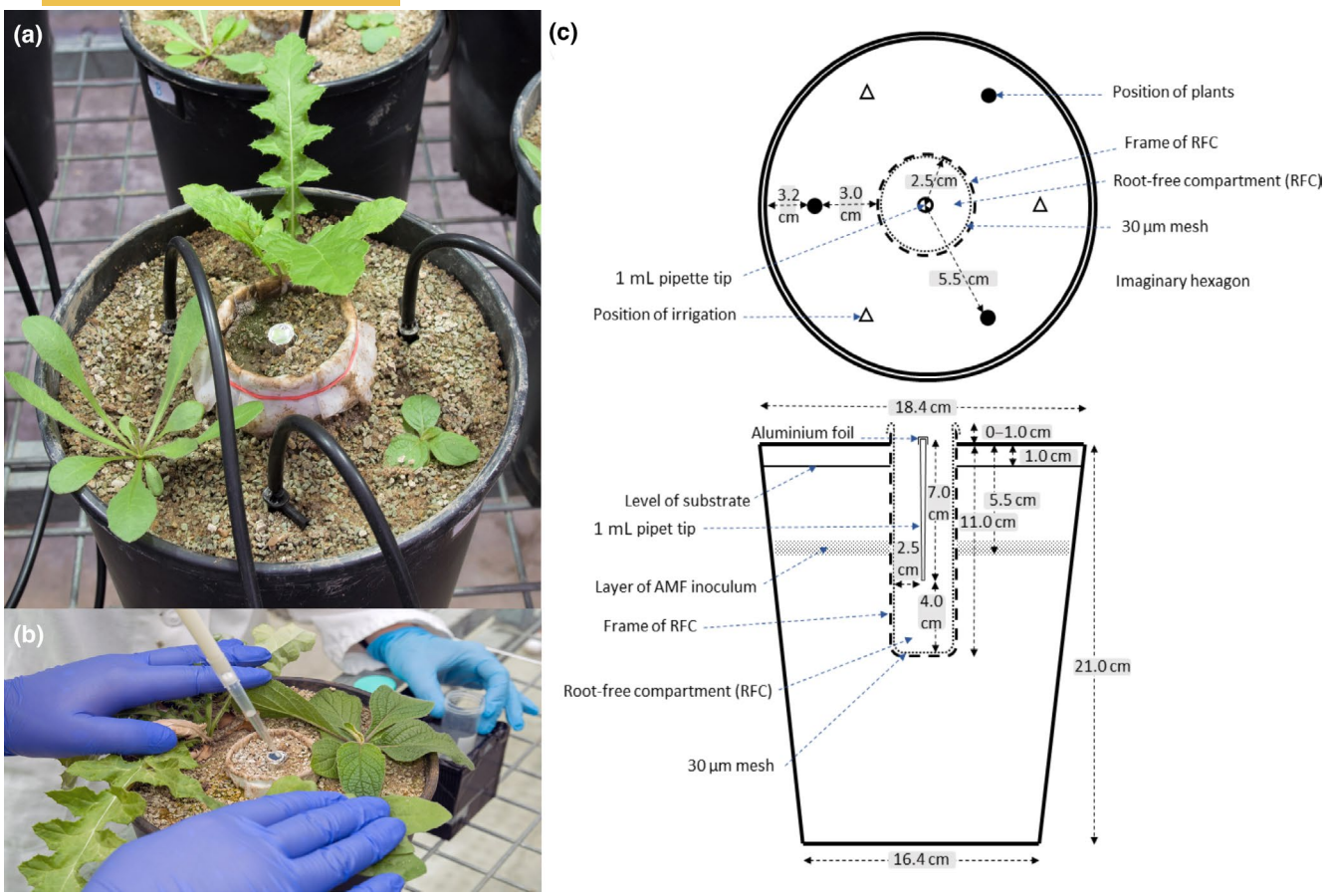

FIGURE 2 Experimental three-species pot with *Echinops sphaerocephalus*, *Inula conyzae* and *Crepis biennis* growing along the perimeter (a, c) and ^{33}P labelling (b).

Slavíková, et al., 2018). Plants grew in the pots at regular intervals (Figure 2a,c) all equidistant from the central RFC (nylon mesh barrier with pores 42 µm in diameter; 5 × 11 cm; Figure 2). A 1-mL pipette tip with a cut end was placed in the centre of the RFC and covered with foil until ^{33}P was later applied into the RFC through this canal (Figure 2a,b).

2.4 | Mycorrhizal inoculation

Pots in the AM fungi inoculated (AMF+) treatment were supplemented with 120 g of mycorrhizal inoculum that consisted of potting substrate containing root fragments of leek (*Allium porrum* L.), which had been used as a host plant for cultivating the widespread generalist *F. mosseae* BEG 161 (BEG is an abbreviation for the International Bank for the Glomeromycota; www.i-beg.eu) sourced from Central Europe. The non-inoculated (AMF–) treatment received 120 g of non-mycorrhizal (mock) inoculum which consisted of potting substrate containing root fragments of leek from a previous pot culture grown under the same conditions and for the same period of time as the AMF+ pot cultures, but without AM fungi. The inocula were added in a layer 4.5 cm beneath the surface of the potting substrate (Figure 2a) before the sowing of experimental plants.

2.5 | Isotope labelling

Five days before harvest, we conducted pulse labelling with $^{13}CO_2$. To prevent photosynthetic $^{13}CO_2$ fixation by unlabelled plants sharing the same pot with the labelled plant, we covered unlabelled plants with thick aluminium foil during the labelling process to keep them in darkness. Each pot had only one species labelled (see experimental design, Figure 1), with five replicate pots, which allowed us to gather information on $^{13}CO_2$ fixation by all co-occurring plant species. The pots were placed into an air-tight Plexiglas chamber (100 × 100 × 85 cm) equipped with a fan to ensure proper mixing of the inner atmosphere, following the procedures of previous studies (Konvalinková et al., 2017; Řezáčová, Slavíková, et al., 2017, 2018; Slavíková et al., 2017). Temperature, photon flux density and CO_2 concentration in the chamber were monitored throughout the labelling using a Testo 435-2 datalogger equipped with an IAQ [indoor air quality] monitor and a lux probe, Testo AG, Lenzkirch, Germany. The $^{13}CO_2$ was introduced to the chamber by adding 60 mL of 20% HCl onto 5 g of $NaH^{13}CO_3$ (>99% ^{13}C, Cambridge Isotopic Laboratories, Inc., Andover, MA, USA). The labelling took 120 min at 152 µmol photons $m^{-2}s^{-1}$ (measured inside the chamber) labelling. Immediately after the $^{13}CO_2$ labelling, carrier-free ^{33}P-phosphoric

acid (Hartmann Analytic GmbH, Braunsweig, Germany; diluted in H_2O) was applied into the pipette tips buried in RFCs within pots (377,980 Bq ^{33}P pot^{-1} decay-corrected for the time of labelling).

2.6 | Plant harvest and sample analyses

One hundred and fourteen days following the establishment of the experiment, shoots of all plants were cut at the shoot–root interface, dried for 3 days at 65°C and weighed to determine shoot dry weight (hereafter called plant biomass), P, ^{33}P, N and ^{13}C. The roots were removed from the substrate, washed under cold tap water, weighed and cut into 1.5 cm fragments. The roots were then subsampled and preserved for either staining and microscopy in the AMF− treatment or for molecular analyses of the AMF+ treatment. Roots for microscopy were immersed in 50% ethanol, while those for molecular analyses were frozen. The remainder of the root system was weighed after clipping, dried for 5 days and weighed again. The fresh to dry weight ratio was determined and total root dry weight was calculated for each root system. Approximately 200 g of representative (thoroughly mixed) substrate per pot was dried at 65°C for 4 days before further determination of ^{13}C in whole cell fatty acids (WCFA) present in the substrate. Dried shoot, root and substrate samples were milled to fine powder using a MM200 ball mill (Retsch, Haan, Germany) prior to further elemental, isotopic and molecular analyses.

We assessed mineral nutrient concentrations of P, ^{33}P and N within shoot tissues. The P concentration in shoot tissues was assessed by colorimetry in HNO_3 (69%) extracts of combusted (550°C, 12 h) samples using the malachite green assay (Ohno & Zibilske, 1991). ^{33}P activity was assessed by scintillation and decay was corrected for the time of labelling as in Konvalinková et al. (2017, Supporting Information to the publication). The N concentration in shoots (results are presented in the Supporting Information only) and isotopic composition of ^{13}C in WCFA and shoots were measured using a Delta V Advantage isotope ratio mass spectrometer (IRMS) as described earlier (Slavíková et al., 2017) and its peripheries (ThermoFisher Scientific, Waltham, MA, USA), including Trace 1310 gas chromatograph as described in Konvalinková et al. (2017) and Řezáčová, Slavíková, et al. (2018). Plant P and N contents (i.e. the amounts of the respective elements in the shoot) were calculated from the measured nutrient concentrations in shoots and multiplied by the dry weights of the shoots. Whole cell fatty acids were extracted from all dried roots and substrate according to the procedure described earlier by Frostegård et al. (1991) but with slight modifications described in Řezáčová, Zemková, et al. (2018). An internal standard (100 µL of nonadecanoic acid, Sigma-Aldrich, Switzerland, dissolved in hexane, 1 g L^{-1}) was added to samples before WCFA extraction. Subsequently, WCFA were transmethylated using the trimethylchlorosilane approach (Welc et al., 2012).

The amount of AMF-signature 16:1ω5 fatty acid was calculated compared to internal standard. Carbon isotopic composition in the plant biomass and WCFA in roots and the substrate was used to calculate ^{13}C derived from the ^{13}C labelling pulse as in Konvalinková et al. (2015) and Řezáčová, Slavíková, et al. (2018). We accounted for natural ^{13}C abundance by subtracting the isotopic composition of tissues and WCFA in unlabelled pots from those in ^{13}C-labelled pots. Thus, *excess* $^{13}C = n \times (F_{samp} - F_{unlab})$, where n was the amount of C (µmol), F_{samp} was the fractional abundance of ^{13}C isotope [$^{13}C/(^{12}C + ^{13}C)$] and F_{unlab} was the averaged fractional abundance of ^{13}C in the respective unlabelled pots. Excess ^{13}C in 16:1ω5 WCFA per gram of roots or substrate was calculated using n and F values of the methyl esters; because of the subtraction of F_{unlab}, this calculation also corrected for the ^{13}C signature distortion by transmethylation, as the same stock of methanol was used for the transmethylation of all samples. Total excess ^{13}C in 16:1ω5 WCFA in roots or substrate was then calculated by multiplying the excess ^{13}C in 16:1ω5 WCFA per gram of sample by root or substrate total dry weight.

We quantified AM fungus presence within roots in two ways. For AMF− treatments, the absence of AM fungal structures in all roots was checked microscopically (one composite sample per M− pot) using the magnified intersection method (McGonigle et al., 1990), scoring 100 magnified (×200) root intersections per sample, after staining the roots with trypan blue (Koske & Gemma, 1989).

To assess AM fungal abundance in the roots of AMF+ plants, DNA was extracted from frozen roots (70–80 mg per sample) using the glass milk method with the CTAB extraction buffer as described in Gryndler et al. (2013). Subsequently, a quantitative real-time PCR (qPCR) was employed to quantify AM fungus abundance in the roots, with a *moss* marker, targeting sequence-specific motif of *F. mosseae* in the nuclear large ribosomal subunit (LSU) genes (Thonar et al., 2012).

2.7 | Statistical analyses

To assess the impact of mycorrhizal inoculation and the presence of *E. sphaerocephalus* on resource allocation within pots, we performed two-way ANOVAs on total biomass produced per pot by native plants and on their per-pot summed shoot P content using *inoculation* and *E. sphaerocephalus presence* as factors. To determine if the presence of *E. sphaerocephalus* altered the CMN-mediated relationship between the two native plant species, we calculated the proportions of plant biomass produced by *I. conyzae* from the biomass produced by both native plants in the cultivation pot (IC/(IC + CB)), as well as the P content proportion in *I. conyzae*'s shoot. Because the remaining part of the particular resource was thus assignable to the *C. biennis*, we further use terms 'IC:CB biomass' and 'IC:CB P content' for the IC proportions from the total. We then carried out two-way ANOVAs with *E. sphaerocephalus presence* and *mycorrhizal inoculation* as factors. We then analysed data from pots with all three species to examine how mycorrhizal inoculation and each plant species contributed to biomass and P content within pots using two-way ANOVAs with *inoculation* and *species* as factors.

We then examined CMN-mediated P uptake using foliar ^{33}P data in inoculated pots. Similar to the biomass and P content analyses, we

examined the total amount of ^{33}P obtained by the native plants in the presence and absence of *E. sphaerocephalus*; because ^{33}P could only be obtained by AM fungi, we conducted a one-way ANOVA with *E. sphaerocephalus presence* as a factor on data from AMF+ pots. We conducted a similar analysis on the proportion of ^{33}P obtained by *I. conyzae* from the total of both native plants in the pot (IC:CB ^{33}P). We then investigated if CMNs distributed ^{33}P differently among all three species in inoculated pots using a one-way ANOVA on the proportion of ^{33}P obtained by each species using *species* as a factor.

To determine how the presence of *E. sphaerocephalus* affected the contribution of ^{13}C to 16:1ω5 WCFA in roots and substrate by the two native species, we took the excess ^{13}C of *I. conyzae* and *C. biennis* and then conducted a two-way ANOVAs with *E. sphaerocephalus presence* and *species* as factors. To determine if the different plant species differ in their ^{13}C provisioning to AM fungus in roots or substrate in inoculated, three species pots, we examined the excess ^{13}C contributed by each species using a one-way ANOVA with *species* as a factor. These analyses were performed on log-transformed values of ^{13}C excess in 16:1ω5 WCFA from roots and substrate.

We assessed how the presence of *E. sphaerocephalus* affected the LSU abundance of each species using a two-way ANOVA with *E. sphaerocephalus presence* and *species* as factors. To determine if the different plant species differ in their LSU abundance in roots in inoculated, three species pots, we examined the LSU abundance by each species using a one-way ANOVA with *species* as a factor. These analyses were performed on log-transformed values of AM fungus LSU abundance in roots.

Analyses of variance were conducted in R (version 3.6.3, R Core Team, 2013, http://www.R-project.org/) after checking for normality and homogenous variance ($\alpha = 0.05$). When appropriate, post hoc comparisons were carried out using Tukey HSD tests. Mean values and standard errors per treatment combination are presented.

3 | RESULTS

3.1 | Plant biomass

The presence of *E. sphaerocephalus* decreased the total biomass produced by the native species per pot (Figure 3A; Table 1) likely because the density of plants increased from two to three plants per pot and *E. sphaerocephalus* was the largest species within pots (Figure 5A; Table 2). Despite the presence of *E. sphaerocephalus*, however, IC:CB biomass remained the same across two and three species pots (Figure 4A; Table 2).

The presence of AM fungi had a significant effect on the total biomass produced by native plants (Table 1), with inoculated pots producing more total biomass than non-inoculated ones (Figure 3A). The IC:CB biomass was significantly affected by inoculation as well (Figure 4a; Table 1). Whether inoculated or not, *I. conyzae* produced a similar amount of biomass (Figure 5A), but *C. biennis* produced more biomass in inoculated treatments (Figure 5A), suggesting that *C. biennis* was more responsive to AM fungi than *I. conyzae*.

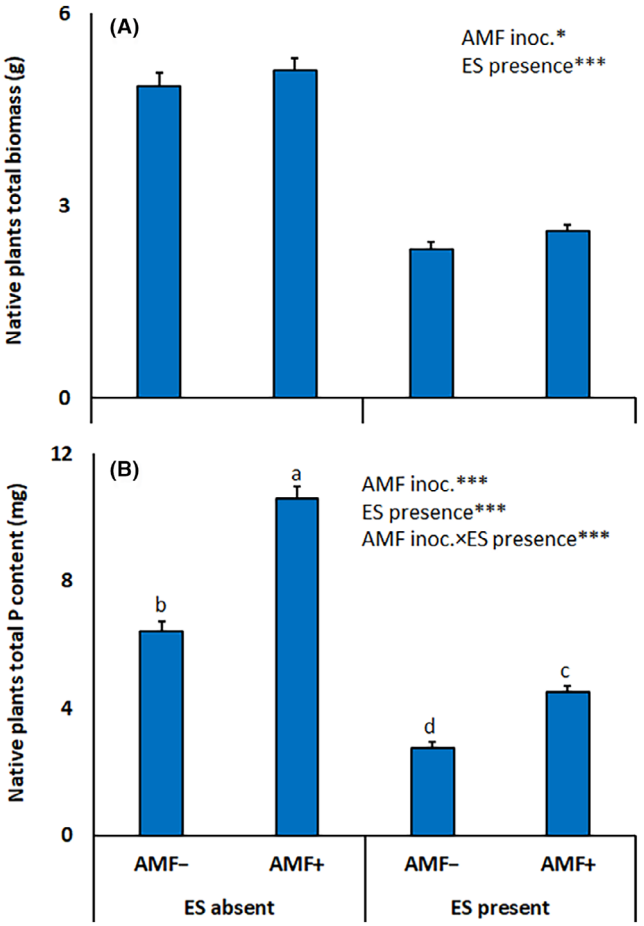

FIGURE 3 The total (per pot summed) shoot biomass (A) and total shoot P content (B) of native plants growing in the same pot, as affected by the presence of *Echinops sphaerocephalus* (ES presence) and mycorrhizal fungus inoculation (AMF+: Inoculated; AMF−: Non-inoculated control). Bars represent means accompanied by standard errors ($n = 15$ for pots where *E. sphaerocephalus* was absent, $n = 20$ for pots where *E. sphaerocephalus* was present). Different letters above individual bars indicate significant differences among means as revealed by the Tukey *post hoc* test ($\alpha = 0.05$).

3.2 | Phosphorus and carbon dynamics

Just as with biomass, the presence of *E. sphaerocephalus* decreased the total P content of native species per pot (Figure 3B; Table 1) and did not affect the IC:CB P content (Figure 4b; Table 1). The presence of AM fungi increased total P content per pot of the native species (Figure 3B, Table 1). An interaction between the presence of *E. sphaerocephalus* and AM fungus inoculation was also detected (Table 1), and the positive effect of AM inoculation on the total P content of native plants was smaller when *E. sphaerocephalus* was present compared to when it was absent. Inoculated native plants in two species pots had the most P content, while non-inoculated native plants in three species pots had the least total P content per pot (Figure 3B).

Just as the IC:CB biomass was reduced by inoculation, the IC:CB P content was decreased in inoculated treatments (Figure 4B;

TABLE 1 The effects of *E. sphaerocephalus* presence, mycorrhizal fungus inoculation and their interaction as revealed by two-way ANOVAs on native plant (*I. conyzae* and *C. biennis*) total shoot biomass (IC:CB Biomass) and total P contents (IC:CB P content) summed per pot, as well as the proportions of *I. conyzae* biomass and P content from the totals produces by both in the cultivation pot.

		Total native plant biomass	Total native plant phosphorus content	IC:CB biomass	IC:CB P content
E. sphaerocephalus presence	$F_{1,66}$	361.1	410.9	0.01	0.5
	p	**<0.0001**	**<0.0001**	0.94	0.49
Inoculation	$F_{1,66}$	4.0	136.6	14.3	70.0
	p	**0.049**	**<0.0001**	**0.0003**	**<0.0001**
E. sphaerocephalus presence × Inoculation	$F_{1,66}$	0.03	24.9	1.7	1.1
	p	0.86	**<0.0001**	0.20	0.31

Note: Significant results are bolded ($\alpha = 0.05$).

TABLE 2 The effect of mycorrhizal fungus inoculation, plant species and their interaction as revealed by two-way ANOVAs on the proportion of shoot dry biomass and shoot phosphorus content of native plants *I. conyzae* and *C. biennis*, and *E. sphaerocephalus* within three-species pots (i.e. the share of resources diverted to each plant within a pot).

	Proportion of biomass		Proportion of P content	
	$F_{2,114}$	p	$F_{2,114}$	p
Inoculation	0.0	1.00	0.0	1.00
Species	441.0	**<0.001**	48.9	**<0.001**
Inoculation × species	6.6	**<0.002**	23.6	**<0.001**

Note: Significant results are bolded ($\alpha = 0.05$).

Table 1). In the *E. sphaerocephalus* presence, the increase in P content proportion gained by *C. biennis* in inoculated pots resulted in these proportions not differing for *C. biennis* and *E. sphaerocephalus*, the plant with largest proportion of biomass (Figure 5A), which resulted in a significant interaction between plant and AMF inoculation (Table 2).

In inoculated pots, the presence of *E. sphaerocephalus* did not affect the total ^{33}P obtained by native plants ($F_{1,23} = 0.002$, $p = 0.96$; absence of *E. sphaerocephalus*: 12.6 ± 1.7 kBq, presence of *E. sphaerocephalus*: 12.5 ± 0.7 kBq) nor the IC:CB ^{33}P ($F_{1,23} = 1.5$, $p = 0.23$; when *E. sphaerocephalus* was absent: 12 ± 3/88 ± 7%; when *E. sphaerocephalus* was present: 22 ± 3/78 ± 7%). However, when examining the proportion of ^{33}P obtained by each species within pots where *E. sphaerocephalus* was present, *C. biennis* obtained the largest proportion of ^{33}P from CMNs (Figure 6A), while *I. conyzae* and *E. sphaerocephalus* obtained a similar, lower proportion of ^{33}P ($F_{2,42} = 23.7$, $p < 0.0001$; Figure 6A).

When *E. sphaerocephalus* was present, less excess ^{13}C originating from native plants was found in 16:1ω5 WCFA in the substrate (Table 3; 1770 ± 322 nmol) than when *E. sphaerocephalus* was absent (4645 ± 1076 nmol). When *E. sphaerocephalus* was absent, *C. biennis* and *I. conyzae* had similar excess ^{13}C in their root 16:1ω5 WCFA.

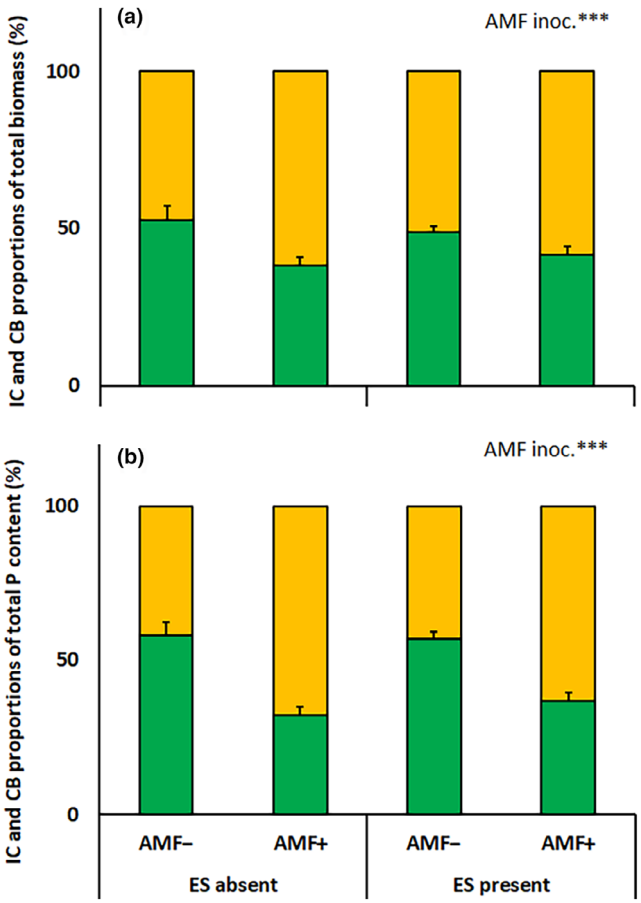

FIGURE 4 The share of resources diverted between native plants per pot as represented by the proportion of shoot dry biomass (a) and shoot P content (b) of *Inula conyzae* (IC, green bars) compared to biomass produced by *C. biennis* (CB, yellow bars) growing in the same pot, as affected by the presence of *Echinops sphaerocephalus* (ES) and mycorrhizal fungus inoculation (AMF+: Inoculated; AMF−: Non-inoculated). Results from a two-way ANOVA are presented for the *I. conyzae* fraction of biomass and P content per pot. Bars represent means accompanied by standard errors ($n = 15$ pots where *E. sphaerocephalus* was absent, $n = 20$ for pots where *E. sphaerocephalus* was present).

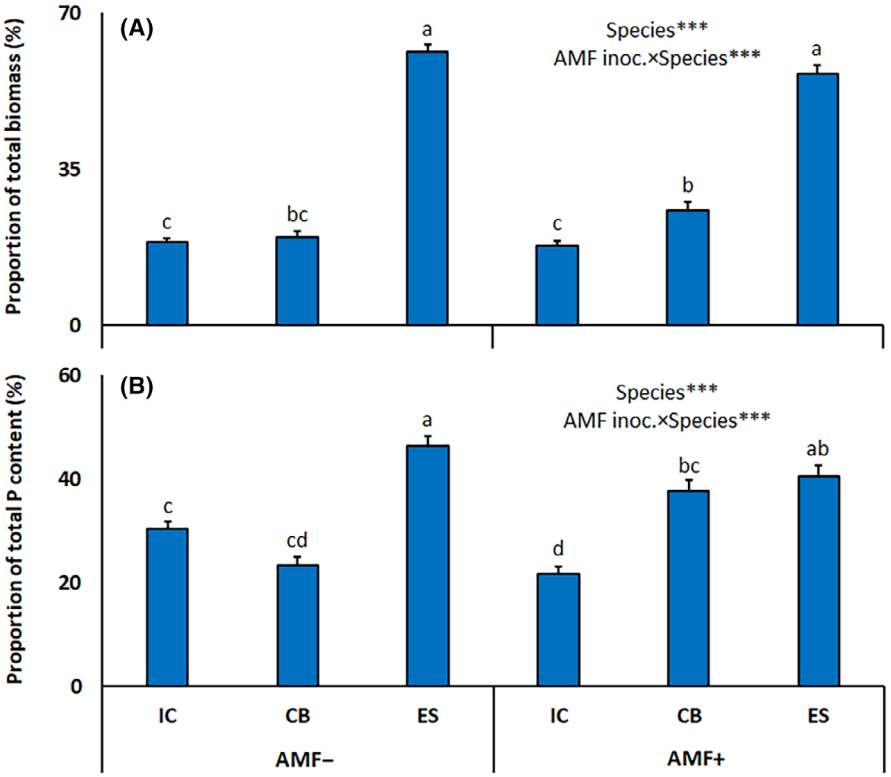

FIGURE 5 Proportions of shoot dry biomass (A) and shoot P content (B) of native plants *Inula conyzae* (IC), *C. biennis* (CB) and *Echinops sphaerocephalus* (ES) growing in the same pot from the total resources summed per pot as affected by mycorrhizal fungus inoculation (AMF+: Inoculated; AMF−: Non-inoculated) and plant species. Bars represent means accompanied by standard errors ($n=20$). Different letters above individual bars indicate significant differences among means as revealed by the Tukey post hoc test ($\alpha=0.05$).

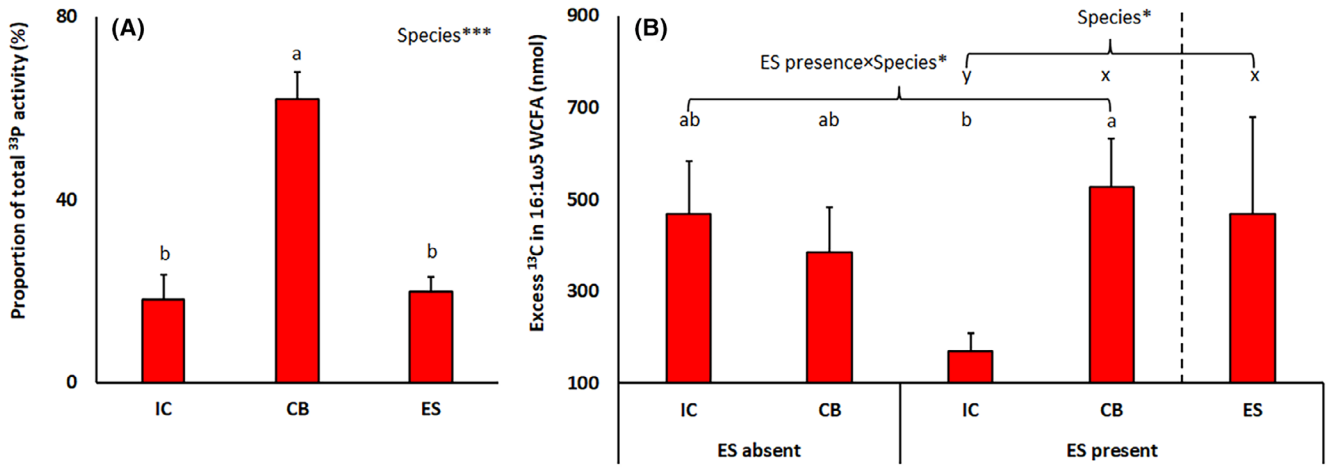

FIGURE 6 Proportions of ^{33}P of the total ^{33}P received by all three species in three-species pots acquired into shoots (A) and excess ^{13}C in 16:1ω5 whole cell fatty acid (WCFA) in roots (B) of co-occurring mycorrhizal fungus inoculated native plants *Inula conyzae* (IC) and *C. biennis* (CB), and *Echinops sphaerocephalus* (ES), as affected by plant species and/or E. *sphaerocephalus* presence. Bars represent means accompanied by standard errors ($n=15$ for ^{33}P data, $n=5$ for ^{13}C data). Different letters above individual bars indicate significant differences among means as revealed by the Tukey post hoc test ($\alpha=0.05$). Statistical analyses were performed on log-transformed values of ^{13}C excess in 16:1ω5 WCFA.

When *E. sphaerocephalus* was present, however, excess ^{13}C of WCFA in *C. biennis* roots was greater than of *I. conyzae* roots ($F_{2,12}=5.5$, $p=0.02$). Notably, the excess ^{13}C of root WCFA was not different between *C. biennis* and *E. sphaerocephalus*, both of which differed from *I. conyzae* (Figure 6B). The effect of plant species on the excess ^{13}C of WCFA found in the substrate in three-species pots, however, was not detectable ($F_{2,12}=0.3$, $p=0.73$).

3.3 | AM fungal abundance in plant roots

No structures of mycorrhizal fungi were present in any non-inoculated treatment as revealed by light microscopy of plant roots. The presence of *E. sphaerocephalus* increased AM fungus abundance the same in both the native plants (Figure 7; $F_{1,66}=6.1$, $p=0.02$); however, *C. biennis* had lower LSU abundance than *I. conyzae*

TABLE 3 The effects of *E. sphaerocephalus* presence, plant species (*C. biennis*, *CB* and *I. conyzae*; *IC*) and their interaction as revealed by two-way ANOVAs, respectively, on the ^{13}C excess in 16:1ω5 whole cell fatty acid (WCFA) in roots and substrate affected by native plants.

		Excess ^{13}C in 16:1ω5 WCFA	
		Roots	Substrate
E. sphaerocephalus presence	$F_{1,16}$	1.2	4.7
	p	0.28	**0.04**
Species	$F_{1,16}$	2.6	0.99
	p	0.12	0.33
E. sphaerocephalus presence × species	$F_{1,16}$	5.2	0.007
	p	**0.04**	0.93

Note: Significant results are stated in bold.

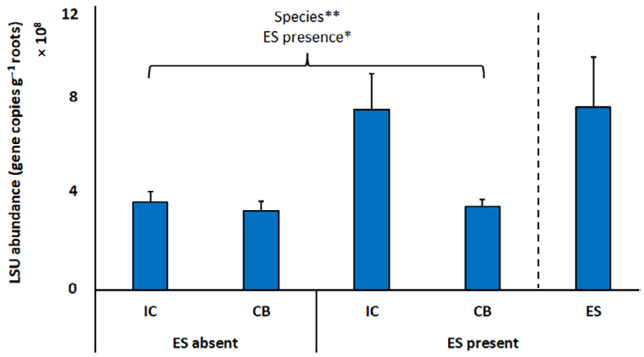

FIGURE 7 LSU abundance in roots of mycorrhizal fungus inoculated native plants *Inula conyzae* (IC), *C. biennis* (CB) and *Echinops sphaerocephalus* (ES), as affected by plant species and by plant species and *E. sphaerocephalus* presence (ES presence). Statistical analyses were performed on log-transformed values of AM fungus LSU abundance. Bars represent means accompanied by standard errors ($n = 15$ for pots where *E. sphaerocephalus* was absent, $n = 20$ for pots where *E. sphaerocephalus* was present).

($F_{1,66} = 9.6$, $p = 0.003$ for pots where *E. sphaerocephalus* was absent; Figure 7), possibly even lower than *E. sphaerocephalus*—this was marginally significant in three species pots ($F_{2,57} = 3.1$, $p = 0.05$).

4 | DISCUSSION

In this study, we investigated how a CMN among three Central European plant species with different origins and abilities to spread mediated plant interactions for P. We found that, in accordance with the initial hypotheses, CMNs played a significant role in below-ground interactions among interconnected plants, and the presence of a third plant within the CMN altered C and P exchange dynamics between native plants and AM fungi. Therefore, the identity and number of plant species affected C-for-P exchange within the community, with the invasive *E. sphaerocephalus* bearing the highest C cost for CMN-acquired P.

4.1 | Factors behind mycorrhizal P redistribution among plants in CMNs

Although the findings of this study did not detect an effect of *E. sphaerocephalus* in CMNs on the morphometric measurements (the relative proportions of biomass produced) of native plants, the use of isotopes revealed that this plant does affect C-for-P trade dynamics within CMNs. When grown in two-species pots, the native plants provided equal amounts of ^{13}C to CMNs, but the presence of *E. sphaerocephalus* resulted in *I. conyzae* provisioning less C to AM fungi than *C. biennis*, while *C. biennis* provisioned a similar amount of C as *E. sphaerocephalus*. The large C transfer, however, did not benefit *E. sphaerocephalus*, which received a similarly small share of ^{33}P as *I. conyzae*. *C. biennis*, though, received three times more ^{33}P than *I. conyzae* and *E. sphaerocephalus*. Ultimately, the cost of P was two to three times more expensive for *E. sphaerocephalus* than *I. conyzae* and *C. biennis*, respectively. This disproportional C investment did not necessarily disadvantage any plant involved in CMNs in biomass production, most likely because C costs of AM fungi can be negligible to main C donors (Figueiredo et al., 2021). Plants allocate an average of 6% of plant assimilated C to mycorrhizae (Hawkins et al., 2023), so it is possible that this increase in C provisioning was not significant enough to affect growth or it did not occur for long enough to affect biomass. This change in C-for-P exchange detected by isotopes in the presence of *E. sphaerocephalus*, however, suggests that *E. sphaerocephalus* can alter interactions for mineral nutrients established among native plants growing together in CMNs.

Our findings are counterintuitive to the predictions of the biological market theory in which plants allocate C preferentially to more beneficial fungal partners, while AM fungi provide nutrients preferentially to photosynthate-rich hosts (Kiers et al., 2011, 2016; Kiers & Denison, 2008; Kiers & van der Heijden, 2006; Wyatt et al., 2014). Although several studies have found evidence for this theory in greenhouse experiments (i.e. Fellbaum et al., 2014; Weremijewicz et al., 2016), others have found unequal C-for-P trade within CMNs interconnecting different plant species (Durant et al., 2023; Lekberg et al., 2024; Walder et al., 2012). It should be noted here that by using only 1 AM fungal species for creating model CMNs to simplify experimental design and feasibility, we have brought into the set-up somewhat artificial nature compared to natural ecosystems and limited the plant's ability to select and interact with the most beneficial symbionts and thus precluded direct testing of the biological market hypothesis from the plant perspective. Distribution of P among plants could be related to the abundance of AM fungi in plant roots, which can correlate with the amount of received P (Treseder, 2013). However, in our study, the AM fungal abundance did not differ significantly for individual plants in three-species communities. Nevertheless, the inequality, in which a large C provisioner receives little mineral nutrients from CMNs in return, can

be attributed to differences in expression of inorganic P (Pi) fungal transporters by different fungal species in the plant's periarbuscular space (Walder et al., 2016). For example, a high affinity Pi transporter was not expressed in *Sorghum bicolor* when it was grown in mixed culture with *Linum usitatissimum* (Walder et al., 2016), likely explaining why *S. bicolor*, despite provisioning most of the C found in a CMN interconnecting it with *L. usitatissimum*, received little P in return (Walder et al., 2012). Thus, single AM fungus species combinations with different plant hosts may affect the C-for-P stoichiometry. Because our experiment investigated a CMN formed by a single species of AM fungi, *F. mosseae*, differences in Pi transporter regulation could have contributed to the unequal trade exhibited by *E. sphaerocephalus* in CMNs. It is likely that by sourcing from the same area, the symbiosis between the native plant species and *F. mosseae* may have coevolved to maximize the exchange of a limiting resource like P (Johnson et al., 2010). In contrast, the differing origins of the non-native plant and its AMF partner could also explain the host plant's inability to harness CMNs to its advantage. Enhanced photosynthetic abilities of *E. sphaerocephalus* might also contribute to its high carbon investment. This is supported by research indicating that invasive plants may allocate more carbon to growth due to reduced investment in defence mechanisms, such as biochemical or physical defences, against native herbivores or pathogens (e.g. Blossey & Notzold, 1995; Huang et al., 2010).

When seeking to uncover the factors contributing to the unequal distribution of P by CMNs among different host plants, we must also consider the mycorrhizal responsiveness of these plants. Mycorrhizal responsiveness, defined as the mean biomass difference of plants grown with and without mycorrhiza, standardized for growth with mycorrhiza (Janos, 2007), reflects plant investment strategies in mycorrhizal associations. Plants with varying mycorrhizal responsiveness are more likely to coexist, as mycorrhiza can serve as a coexistence mechanism, balancing and stabilizing forces (Chesson, 2000; Wagg et al., 2011). Non-native invaders, unlike perennials, are generally annuals representing an R strategy and are typically facultative mycorrhizal (Richardson et al., 2000; Vogelsang & Bever, 2009). Such plants establish weaker associations with AM fungi and often exhibit low levels of AM fungal root colonization (Lin et al., 2015; Richardson et al., 2000; Vogelsang & Bever, 2009). However, this was not the case of *E. sphaerocephalus*, similar to the other two plants involved, is biennial to perennial and was comparably colonized by AM fungi. Moreover, based on the previous research (Řezáčová, Slavíková, et al., 2017, 2018), *E. sphaerocephalus* was the most mycorrhiza-responsive species in our experiment, and based on the results of this research, *C. biennis* is more mycorrhiza-responsive than *I. conyzae*, which was the least responsive plant. Our results demonstrate that CMNs was more beneficial for the less-mycorrhiza responsive plants, *I. conyzae* and *C. biennis*, and less beneficial for more mycorrhiza-responsive *E. sphaerocephalus* in the three-plant community. These findings suggest that the more responsive a plant to mycorrhiza is, the weaker its advantage through CMNs.

The fact that *E. sphaerocephalus* can alter proportions of exchanged C for P established among native plants growing together in CMNs point out to the fact that perhaps any additional plant can change the dynamics of resource exchange between plants and CMNs. This suggests that CMNs mediated supply to its hosts may not be based on C for P exchanges with specific individuals, but driven by source and sink strengths across plant communities. This highlights the potential importance of plant neighbours in CMNs in the dynamics of resource exchange and is consistent with the findings of Durant et al. (2023).

4.2 | The role of native CMNs in the spread of invasive *E. sphaerocephalus*

In light of the fact that our model community consisted of an invader and two native plants, it is possible to assess the influence of *E. sphaerocephalus*, on CMNs-mediated relationships with respect to the mechanisms influencing its invasiveness. The introduction of the invasive *E. sphaerocephalus* into our model plant community changed the C cost ratio between the native plants, which altered the settled coexistence of *I. conyzae* and the native *C. biennis*. Notably, we have not yet found a similar experiment, which examines the influence of a third plant on below-ground relationships within CMNs by altering resource flows among the involved partners in the scientific literature. Given the high cost of mycorrhizal P acquisition for invasive *E. sphaerocephalus*, it is possible that it acquired some of the mycorrhizal C cost from native plants, especially *I. conyzae*. While the hypothesis that *E. sphaerocephalus* could exploit CMNs to gain benefits at the expense of native plants was not confirmed, the invader's strategy may still be linked to destabilizing below-ground relationships among native plants, although it did not manifest in our experiment on biomass. It is worth noting that, for a plant of *E. sphaerocephalus* size, assuming the C cost is typically below 10% (but usually much lower) of the photosynthetically assimilated C (Řezáčová, Konvalinková, et al., 2017; Slavíková et al., 2017), taking over a portion of these payments may not be a significant burden.

Two hypotheses have been postulated to explain the relationship between AM fungi and invasive plants, and *E. sphaerocephalus* does not fit into either hypothesis. The first, known as the enhanced mutualism hypothesis (Reinhart & Callaway, 2006), suggests that AM fungi enhance the competitiveness of invasive plants. The second, the degraded mutualism hypothesis, assumes that invasive plants weaken native plants by disrupting mycorrhizal associations, assuming invasive plants do not associate with AM fungi (Vogelsang & Bever, 2009). In the case of the invasive *E. sphaerocephalus*, contrary to the degraded mutualism hypothesis, it does form mycorrhizal associations. However, in contrast to the enhanced mutualism hypothesis (and previous research of Řezáčová et al. (2020)), AM fungi and their CMNs did not significantly support the plant. Instead, by supplying C to native CMNs, *E. sphaerocephalus* destabilized relationships between native plants. While the involvement of native CMNs may have benefited the spread of invasive *E. sphaerocephalus*, it did not entirely align with the enhanced mutualism hypothesis.

However, prior studies on the role of AM fungi/CMNs in the spread of invasive plants did not include the flow of nutrients and C within a community of three plant species, thus limiting their ability to investigate changes in below-ground relationships among native plants following the introduction of an invader.

4.3 | Methodological issues

One question to address is whether the plants in the community were indeed connected by CMNs or whether we only observed an individual plant's relationships with AM fungi. Proving this connection is typically challenging. However, given that the presence of *E. sphaerocephalus* increased colonization of both native plants (and increased it equally in both) while reducing the amount of C supplied by the native plants to the fungus, we hypothesize that the colonization of native plants increased due to the C contributions by *E. sphaerocephalus* to CMNs, thereby interconnecting all three plants through CMNs.

We used ^{13}C pulse-chase labelling as AMF receive the recently fixed photosynthates from their hosts, using it for their growth, respiration and biological maintenance (Jansa et al., 2011). Although we want to acknowledge the limitations of this approach—a single snapshot offers only a glimpse into the system's function at a specific time and may not reflect the full range of exchange rates that occur over extended periods—it can still reveal valuable insights in assessing the C transfers within the CMN-plants system for a particular growth stage, especially in short-living plant species involved in this study.

5 | CONCLUSIONS

Arbuscular mycorrhiza involves many interactions, particularly when CMNs interconnect roots of multiple plant species. Understanding the trading and distribution of resources between CMNs and their interconnected host plants remains a significant challenge. To date, most studies on CMNs and mineral nutrient/C exchange have involved at most two plant species. It is essential to elucidate the functioning of mycorrhizal symbiosis in a broader context, which becomes especially complex when involving multiple plant species of diverse origins. The inclusion of three plant species in this study, compared to previous research, is novel and allowed us to confirm that resource exchange is largely influenced by the specific plant partners involved in the CMN, not solely by the specifics of the interacting plant and fungus.

In this study, we investigated how three plant species, sharing a similar life strategy but differing in their geographic origin and rate of spread, compete for P from native one-fungus CMNs. Within this model plant community, CMNs sourced from Central Europe exhibited a disproportionate distribution of P among plants, contradicting the biological market theory.

While this is a model and a greatly simplified, it nevertheless also advances our understanding of the role of mycorrhizal fungi in plant invasions, even if only for a single species. CMNs supported plants of the same geographical origin; however, the benefits of mycorrhizal associations imposed costs on the invasive *E. sphaerocephalus*, a plant with the highest mycorrhizal responsiveness. Despite these costs, the invasive plant still derived P benefits from the CMNs and, moreover, destabilized the relationships between native plants by altering the size of the C cost for mycorrhizal P uptake more in one of the native plants. This suggests that the invader's interaction with native CMNs may play a role in its spread.

Our research underscores the importance of integrating mycorrhizal ecology into the study of plant community dynamics to gain a more comprehensive understanding of how these communities are maintained. This approach can also provide insights into the mechanisms driving the expansion of certain invasive plant species. Furthermore, considering that both the plant species and the identity/genotype of the AM fungi can significantly influence the allocation of C in mycorrhizal associations, we advocate for the inclusion of a wider range of mycorrhizal plant species and a consortium of AM fungi in studies focusing on carbon flows within mycorrhizal networks. This broader approach would facilitate the formulation of more generalizable conclusions and enhance our understanding of the complexities of mycorrhizal interactions in diverse plant communities.

AUTHOR CONTRIBUTIONS

Veronika Řezáčová and Tereza Konvalinková planned, designed and carried out the experiment. Veronika Řezáčová and Tereza Konvalinková analysed the data and Veronika Řezáčová and Joanna Weremijewicz wrote the manuscript. All authors approved the final version of the manuscript.

ACKNOWLEDGEMENTS

This research was supported by the Czech Science Foundation (project 18-01486S), the Ministry of Agriculture of the Czech Republic (institutional support MZE-RO0423) and the National Science Foundation Faculty Early Career Development Program (CAREER) grant (#2145142).

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

Data are archived in Zenodo: https://doi.org/10.5281/zenodo.14234259 (Mayerová & Řezáčová, 2024).

ORCID

Veronika Řezáčová https://orcid.org/0000-0002-1749-0355

REFERENCES

Awaydul, A., Xiao, J., Chen, X., Koide, R., Yuan, Y. G., & Cheng, L. (2023). Distribution of N and recently fixed C among a common mycorrhizal network linking an invasive plant, and a native plant. *Functional Ecology*, *37*(9), 2338–2346. https://doi.org/10.1111/1365-2435.14392

Awaydul, A., Zhu, W. Y., Yuan, Y. G., Xiao, J., Hu, H., Chen, X., Koide, R. T., & Cheng, L. (2019). Common mycorrhizal networks influence the distribution of mineral nutrients between an invasive plant, *Solidago canadensis*, and a native plant, Kummerowa striata. *Mycorrhiza*, *29*(1), 29–38. https://doi.org/10.1007/s00572-018-0873-5

Bago, B., Pfeffer, P. E., & Shachar-Hill, Y. (2000). Carbon metabolism and transport in arbuscular mycorrhizas. *Plant Physiology*, *124*(3), 949–957. https://doi.org/10.1104/pp.124.3.949

Bennett, J. A., Maherali, H., Reinhart, K. O., Lekberg, Y., Hart, M. M., & Klironomos, J. (2017). Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science*, *355*(6321), 181–184. https://doi.org/10.1126/science.aai8212

Blossey, B., & Notzold, R. (1995). Evolution of increased competitive ability in invasive nonindigenous plants: A hypothesis. *Journal of Ecology*, *83*(5), 887–889. https://doi.org/10.2307/2261425

Callaway, R. M., Newingham, B., Zabinski, C. A., & Mahall, B. E. (2001). Compensatory growth and competitive ability of an invasive weed are enhanced by soil fungi and native neighbours. *Ecology Letters*, *4*(5), 429–433. https://doi.org/10.1046/j.1461-0248.2001.00251.x

Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics*, *31*, 343–366. https://doi.org/10.1146/annurev.ecolsys.31.1.343

Durant, E., Hoysted, G. A., Howard, N., Sait, S. M., Childs, D. Z., Johnson, D., & Field, K. J. (2023). Herbivore-driven disruption of arbuscular mycorrhizal carbon-for-nutrient exchange is ameliorated by neighboring plants. *Current Biology*, *33*(12), 2566–2573. https://doi.org/10.1016/j.cub.2023.05.033

Faghihinia, M., & Jansa, J. (2022). Mycorrhiza governs plant-plant interactions through preferential allocation of shared nutritional resources: A triple (^{13}C, ^{15}N and ^{33}P) labeling study. *Frontiers in Plant Science*, *13*, 1047270. https://doi.org/10.3389/fpls.2022.1047270

Fellbaum, C. R., Mensah, J. A., Cloos, A. J., Strahan, G. E., Pfeffer, P. E., Kiers, E. T., & Bücking, H. (2014). Fungal nutrient allocation in common mycorrhizal networks is regulated by the carbon source strength of individual host plants. *New Phytologist*, *203*(2), 646–656. https://doi.org/10.1111/nph.12827

Figueiredo, A. F., Boy, J., & Guggenberger, G. (2021). Common mycorrhizae network: A review of the theories and mechanisms behind underground interactions. *Frontiers in Fungal Biology*, *2*, 735299. https://doi.org/10.3389/ffunb.2021.735299

Frostegård, Å., Tunlid, A., & Bååth, E. (1991). Microbial biomass measured as total lipid phosphate in soils of different organic content. *Journal of Microbiological Methods*, *14*(3), 151–163. https://doi.org/10.1016/0167-7012(91)90018-L

Gryndler, M., Trilcova, J., Hrselova, H., Streiblova, E., Gryndlerova, H., & Jansa, J. (2013). Tuber aestivum Vittad. mycelium quantified: Advantages and limitations of a qPCR approach. *Mycorrhiza*, *23*(5), 341–348. https://doi.org/10.1007/s00572-012-0475-6

Hammer, E. C., Pallon, J., Wallander, H., & Olsson, P. A. (2011). Tit for tat? A mycorrhizal fungus accumulates phosphorus under low plant carbon availability. *FEMS Microbiology Ecology*, *76*(2), 236–244. https://doi.org/10.1111/j.1574-6941.2011.01043.x

Hawkins, H. J., Cargill, R. I. M., van Nuland, M. E., Hagen, S. C., Field, K. J., Sheldrake, M., Soudzilovskaia, N. A., & Kiers, E. T. (2023). Mycorrhizal mycelium as a global carbonpool. *Current Biology*, *33*, R560–R573.

Hewitt, E. J. (1966). Sand and water culture methods used in study of plant nutrition. *Technical Communication*, *22*, 431–432.

Huang, W., Siemann, E., Wheeler, G. S., Zou, J., Carrillo, J., & Ding, J. (2010). Resource allocation to defence and growth are driven by different responses to generalist and specialist herbivory in an invasive plant. *Journal of Ecology*, *98*(5), 1157–1167.

Janos, D. P. (2007). Plant responsiveness to mycorrhizas differs from dependence upon mycorrhizas. *Mycorrhiza*, *17*(2), 75–91. https://doi.org/10.1007/s00572-006-0094-1

Jansa, J., Finlay, R. D., Wallander, H., Smith, F. A., & Smith, S. E. (2011). Role of mycorrhizal symbioses in phosphorus cycling. In E. Bunemann, A. Oberson, & E. Frossard (Eds.), *Phosphorus in action. Biological Processes in soil phosphorus cycling* (pp. 137–168). Springer.

Johnson, N. C., & Graham, J. H. (2013). The continuum concept remains a useful framework for studying mycorrhizal functioning. *Plant and Soil*, *363*(1–2), 411–419. https://doi.org/10.1007/s11104-012-1406-1

Johnson, N. C., Wilson, G. W. T., Bowker, M. A., Wilson, J. A., & Miller, R. M. (2010). Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(5), 2093–2098. https://doi.org/10.1073/pnas.0906710107

Khalil, S., Loynachan, T. E., & Tabatabai, M. A. (1994). Mycorrhizal dependency and nutrient-uptake by improved and unimproved corn and soybean cultivars. *Agronomy Journal*, *86*(6), 949–958. https://doi.org/10.2134/agronj1994.00021962008600060005x

Kiers, E. T., & Denison, R. F. (2008). Sanctions, cooperation, and the stability of plant-rhizosphere mutualisms. *Annual Review of Ecology, Evolution, and Systematics*, *39*, 215–236. https://doi.org/10.1146/annurev.ecolsys.39.110707.173423

Kiers, E. T., Duhamel, M., Beesetty, Y., Mensah, J. A., Franken, O., Verbruggen, E., Fellbaum, C. R., Kowalchuk, G. A., Hart, M. M., Bago, A., Palmer, T. M., West, S. A., Vandenkoornhuyse, P., Jansa, J., & Bücking, H. (2011). Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science*, *333*(6044), 880–882. https://doi.org/10.1126/science.1208473

Kiers, E. T., & van der Heijden, M. G. A. (2006). Mutualistic stability in the arbuscular mycorrhizal symbiosis: Exploring hypotheses of evolutionary cooperation. *Ecology*, *87*(7), 1627–1636. https://doi.org/10.1890/0012-9658(2006)87[1627:MSITAM]2.0.CO;2

Kiers, E. T., West, S. A., Wyatt, G. A. K., Gardner, A., Bucking, H., & Werner, G. D. A. (2016). Misconceptions on the application of biological market theory to the mycorrhizal symbiosis. *Nature Plants*, *2*(5), 16063. https://doi.org/10.1038/nplants.2016.63

Konvalinková, T., Puschel, D., Janoušková, M., Gryndler, M., & Jansa, J. (2015). Duration and intensity of shade differentially affects mycorrhizal growth- and phosphorus uptake responses of *Medicago truncatula*. *Frontiers in Plant Science*, *6*, 65. https://doi.org/10.3389/fpls.2015.00065

Konvalinková, T., Puschel, D., Řezáčová, V., Gryndlerova, H., & Jansa, J. (2017). Carbon flow from plant to arbuscular mycorrhizal fungi is reduced under phosphorus fertilization. *Plant and Soil*, *419*(1–2), 319–333. https://doi.org/10.1007/s11104-017-3350-6

Koske, R. E., & Gemma, J. N. (1989). A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research*, *92*, 486–505. https://doi.org/10.1016/S0953-7562(89)80195-9

Lanfranco, L., Bonfante, P., & Genre, A. (2016). The mutualistic interaction between plants and arbuscular mycorrhizal fungi. *Microbiology Spectrum*, *4*(6), 1–20. https://doi.org/10.1128/microbiolspec.funk-0012-2016

Lekberg, Y., Hammer, E. C., & Olsson, P. A. (2010). Plants as resource islands and storage units—Adopting the mycocentric view of arbuscular mycorrhizal networks. *FEMS Microbiology Ecology*, *74*(2), 336–345. https://doi.org/10.1111/j.1574-6941.2010.00956.x

Lekberg, Y., Jansa, J., Mcleod, M., Dupre, M. E., Holben, W. E., Johnson, D., Koide, R. T., Shaw, A., Zabinski, C., & Aldrich-Wolfe, L. (2024). Carbon and phosphorus exchange rates in arbuscular mycorrhizas depend on environmental context and differ among co-occurring

plants. *New Phytologist*, *242*, 1576–1588. https://doi.org/10.1111/nph.19501

Liao, H. X., Huang, F. F., Li, D. J., Kang, L. Y., Chen, B. M., Zhou, T., & Peng, S. L. (2018). Soil microbes regulate forest succession in a subtropical ecosystem in China: Evidence from a mesocosm experiment. *Plant and Soil*, *430*(1–2), 277–289. https://doi.org/10.1007/s11104-018-3733-3

Lin, G. G., McCormack, M. L., & Guo, D. L. (2015). Arbuscular mycorrhizal fungal effects on plant competition and community structure. *Journal of Ecology*, *103*(5), 1224–1232. https://doi.org/10.1111/1365-2745.12429

Mayerová, M., & Řezáčová, V. (2024). *Database of weed species under specific herbicide management* [Data set]. Zenodo. https://doi.org/10.5281/zenodo.14265086

McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L., & Swan, J. A. (1990). A new method which gives an objective-measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. *New Phytologist*, *115*(3), 495–501. https://doi.org/10.1111/j.1469-8137.1990.tb00476.x

Merrild, M. P., Ambus, P., Rosendahl, S., & Jakobsen, I. (2013). Common arbuscular mycorrhizal networks amplify competition for phosphorus between seedlings and established plants. *New Phytologist*, *200*(1), 229–240. https://doi.org/10.1111/nph.12351

Montesinos-Navarro, A., Verdu, M., Querejetac, J. I., Sortibran, L., & Valiente-Banuet, A. (2016). Soil fungi promote nitrogen transfer among plants involved in long-lasting facilitative interactions. *Perspectives in Plant Ecology, Evolution and Systematics*, *18*, 45–51. https://doi.org/10.1016/j.ppees.2016.01.004

Muller, L. M. (2021). Underground connections: Arbuscular mycorrhizal fungi influence on interspecific plant-plant interactions. *Plant Physiology*, *187*(3), 1270–1272. https://doi.org/10.1093/plphys/kiab397

Ohno, T., & Zibilske, L. M. (1991). Determination of low concentrations of phosphorus in soil extracts using malachite green. *Soil Science Society of America Journal*, *55*(3), 892–895. https://doi.org/10.2136/sssaj1991.03615995005500030046x

Pfeffer, P. E., Douds, D. D., Becard, G., & Shachar-Hill, Y. (1999). Carbon uptake and the metabolism and transport of lipids in an arbuscular mycorrhiza. *Plant Physiology*, *120*(2), 587–598. https://doi.org/10.1104/pp.120.2.587

R Core Team. (2013). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Reinhart, K. O., & Callaway, R. M. (2006). Soil biota and invasive plants. *New Phytologist*, *170*(3), 445–457. https://doi.org/10.1111/j.1469-8137.2006.01715.x

Řezáčová, V., Konvalinková, T., & Jansa, J. (2017). Carbon fluxes in mycorrhizal plants. In A. Varma, R. Prasad, & N. Tuteja (Eds.), *Mycorrhiza—Eco-physiology, secondary metabolites, nanomaterials* (pp. 1–21). Springer International Publishing. https://doi.org/10.1007/978-3-319-57849-1_1

Řezáčová, V., Řezáč, M., Gryndler, M., Hršelová, H., Gryndlerová, H., & Michalová, T. (2021). Plant invasion alters community structure and decreases diversity of arbuscular mycorrhizal fungal communities. *Applied Soil Ecology*, *167*, 104039. https://doi.org/10.1016/j.apsoil.2021.104039

Řezáčová, V., Řezáč, M., Gryndlerová, H., Wilson, G. W. T., & Michalová, T. (2020). Arbuscular mycorrhizal fungi favor invasive *Echinops sphaerocephalus* when grown in competition with native *Inula conyzae*. *Scientific Reports*, *10*(1), 20287. https://doi.org/10.1038/s41598-020-77030-0

Řezáčová, V., Řezáč, M., Líblová, Z., Michalová, T., & Heneberg, P. (2021). Stable colonization of native plants and early invaders by arbuscular mycorrhizal fungi after exposure to recent invaders from the Asteraceae family. *Invasive Plant Science and Management*, *14*(3), 147–155. https://doi.org/10.1017/inp.2021.17

Řezáčová, V., Řezáč, M., Wilson, G. W. T., & Michalová, T. (2022). Arbuscular mycorrhiza can be disadvantageous for weedy annuals in competition with paired perennial plants. *Scientific Reports*, *12*(1), 20703. https://doi.org/10.1038/s41598-022-24669-6

Řezáčová, V., Slavíková, R., Konvalinková, T., Hujslová, M., Gryndlerová, H., Gryndler, M., Puschel, D., & Jansa, J. (2017). Imbalanced carbon-for-phosphorus exchange between European arbuscular mycorrhizal fungi and non-native *Panicum* grasses—A case of dysfunctional symbiosis. *Pedobiologia*, *62*, 48–55. https://doi.org/10.1016/j.pedobi.2017.05.004

Řezáčová, V., Slavíková, R., Zemková, L., Konvalinková, T., Procházková, V., Sťovíček, V., Hršelová, H., Beskid, O., Hujslová, M., Gryndlerová, H., Gryndler, M., Püschel, D., & Jansa, J. (2018). Mycorrhizal symbiosis induces plant carbon reallocation differently in C_3 and C_4 *Panicum* grasses. *Plant and Soil*, *425*(1–2), 441–456. https://doi.org/10.1007/s11104-018-3606-9

Řezáčová, V., Zemková, L., Beskid, O., Puschel, D., Konvalinková, T., Hujslová, M., Slavíková, R., & Jansa, J. (2018). Little cross-feeding of the mycorrhizal networks shared between C_3-*Panicum bisulcatum* and C_4-*Panicum maximum* under different temperature regimes. *Frontiers in Plant Science*, *9*, 449. https://doi.org/10.3389/fpls.2018.00449

Richardson, D. M., Allsopp, N., D'Antonio, C. M., Milton, S. J., & Rejmanek, M. (2000). Plant invasions—The role of mutualisms. *Biological Reviews*, *75*(1), 65–93. https://doi.org/10.1017/s0006323199005435

Selosse, M. A., Richard, F., He, X. H., & Simard, S. W. (2006). Mycorrhizal networks: Des liaisons dangereuses? *Trends in Ecology & Evolution*, *21*(11), 621–628. https://doi.org/10.1016/j.tree.2006.07.003

Slavíková, R., Puschel, D., Janoušková, M., Hujslová, M., Konvalinková, T., Gryndlerová, H., Gryndler, M., Weiser, M., & Jansa, J. (2017). Monitoring CO_2 emissions to gain a dynamic view of carbon allocation to arbuscular mycorrhizal fungi. *Mycorrhiza*, *27*(1), 35–51. https://doi.org/10.1007/s00572-016-0731-2

Smith, S. E., & Read, D. J. (2008). *Mycorrhizal symbiosis* (3rd ed., pp. 1–787). Academic Press. https://doi.org/10.1016/B978-0-12-370526-6.X5001-6

Spatafora, J. W., Chang, Y., Benny, G. L., Lazarus, K., Smith, M. E., Berbee, M. L., Bonito, G., Corradi, N., Grigoriev, I., Gryganskyi, A., James, T. Y., O'Donnell, K., Roberson, R. W., Taylor, T. N., Uehling, J., Vilgalys, R., White, M. M., & Stajich, J. E. (2016). A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia*, *108*(5), 1028–1046. https://doi.org/10.3852/16-042

Štajerová, K., Šmilauerová, M., & Šmilauer, P. (2009). Arbuscular mycorrhizal symbiosis of herbaceous invasive neophytes in The Czech Republic. *Preslia*, *81*(4), 341–355.

Thonar, C., Erb, A., & Jansa, J. (2012). Real-time PCR to quantify composition of arbuscular mycorrhizal fungal communities marker design, verification, calibration and field validation. *Molecular Ecology Resources*, *12*(2), 219–232. https://doi.org/10.1111/j.1755-0998.2011.03086.x

Treseder, K. K. (2013). The extent of mycorrhizal colonization of roots and its influence on plant growth and phosphorus content. *Plant and Soil*, *371*(1–2), 1–13. https://doi.org/10.1007/s11104-013-1681-5

van der Heijden, M. G. A., Martin, F. M., Selosse, M. A., & Sanders, I. R. (2015). Mycorrhizal ecology and evolution: The past, the present, and the future. *New Phytologist*, *205*(4), 1406–1423. https://doi.org/10.1111/nph.13288

Vogelsang, K. M., & Bever, J. D. (2009). Mycorrhizal densities decline in association with nonnative plants and contribute to plant invasion. *Ecology*, *90*(2), 399–407. https://doi.org/10.1890/07-2144.1

Wagg, C., Jansa, J., Stadler, M., Schmid, B., & van der Heijden, M. G. A. (2011). Mycorrhizal fungal identity and diversity relaxes plant-plant competition. *Ecology*, *92*(6), 1303–1313. https://doi.org/10.1890/10-1915.1

Walder, F., Boller, T., Wiemken, A., & Courty, P. E. (2016). Regulation of plants' phosphate uptake in common mycorrhizal networks: Role of intraradical fungal phosphate transporters. *Plant Signaling & Behavior*, *11*, 2. https://doi.org/10.1080/15592324.2015.1131372

Walder, F., Niemann, H., Natarajan, M., Lehmann, M. F., Boller, T., & Wiemken, A. (2012). Mycorrhizal networks: Common goods of plants shared under unequal terms of trade. *Plant Physiology*, *159*(2), 789–797. https://doi.org/10.1104/pp.112.195727

Walder, F., & van der Heijden, M. G. A. (2015). Regulation of resource exchange in the arbuscular mycorrhizal symbiosis. *Nature Plants*, *1*(11), 15159. https://doi.org/10.1038/nplants.2015.159

Welc, M., Buenemann, E. K., Fliessbach, A., Frossard, E., & Jansa, J. (2012). Soil bacterial and fungal communities along a soil chronosequence assessed by fatty acid profiling. *Soil Biology & Biochemistry*, *49*, 184–192. https://doi.org/10.1016/j.soilbio.2012.01.032

Weremijewicz, J., & Janos, D. P. (2013). Common mycorrhizal networks amplify size inequality in *Andropogon gerardii* monocultures. *New Phytologist*, *198*(1), 203–213. https://doi.org/10.1111/nph.12125

Weremijewicz, J., Sternberg, L., & Janos, D. P. (2016). Common mycorrhizal networks amplify competition by preferential mineral nutrient allocation to large host plants. *New Phytologist*, *212*(2), 461–471. https://doi.org/10.1111/nph.14041

Weremijewicz, J., Sternberg, L., & Janos, D. P. (2018). Arbuscular common mycorrhizal networks mediate intra- and interspecific interactions of two prairie grasses. *Mycorrhiza*, *28*(1), 71–83. https://doi.org/10.1007/s00572-017-0801-0

Wipf, D., Krajinski, F., van Tuinen, D., Recorbet, G., & Courty, P. E. (2019). Trading on the arbuscular mycorrhiza market: From arbuscules to common mycorrhizal networks. *New Phytologist*, *223*(3), 1127–1142. https://doi.org/10.1111/nph.15775

Workman, R. E., & Cruzan, M. B. (2016). Common mycelial networks impact competition in an invasive grass. *American Journal of Botany*, *103*(6), 1041–1049. https://doi.org/10.3732/ajb.1600142

Wyatt, G. A. K., Kiers, E. T., Gardner, A., & West, S. A. (2014). A biological market analysis of the plant-mycorrhizal symbiosis. *Evolution*, *68*(9), 2603–2618. https://doi.org/10.1111/evo.12466

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. The effects of mycorrhizal fungus inoculation, plant species and presence of *Echinops sphaerocephalus* (ES) on shoot N content of the two native plants (*I. conyzae* and *C. biennis*) growing in two- and three-species pots.

Table S2. The effects of mycorrhizal fungus inoculation and plant species on shoot N content of the three plants (*E. sphaerocephalus*, *Inula conyzae* and *Crepis biennis*) growing in the same three species pots.

Table S3. Raw data.

Figure S1. Shoot N content of (a) plants growing in three-species pots as affected by mycorrhizal fungus inoculation (AMF+: inoculated; AMF−: non-inoculated control) and plant species, (b) native plants, *I. conyzae* (IC) and *C. biennis* (CB), as affected by mycorrhizal inoculation, plant species and *E. sphaerocephalus* (ES) presence.

How to cite this article: Řezáčová, V., Weremijewicz, J., & Michalová, T. (2024). Nutrient exchange within common mycorrhizal networks is altered in a multispecies environment. *Functional Ecology*, *00*, 1–14. https://doi.org/10.1111/1365-2435.14723