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BRIEF REPORT

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The root-associated arbuscular mycorrhizal fungal assemblages of exotic alien plants are simplified in invaded distribution ranges, but dominant species are retained: A trans-continental perspective

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Abstract

Arbuscular mycorrhizal fungi (AMF) provide crucial support for the establishment of plants in novel environments. We hypothesized that the OTU/genus richness and diversity of soil- and root-associated AMF associated with alien plant species in their exotic ranges are lower than those in their native ranges. We examined the root-associated and soil-dwelling AMF of 11 invasive plant species in their native and exotic ranges in the United States and Europe by DNA sequencing of the ITS2 locus. Examined root-associated AMF assemblages were simplified, which manifested as the loss of several AMF genera in the exotic ranges of the plants. These fungal assemblages were also characterized by greater dominance and simplification of the fungal assemblages. The dominant fungal genera were present regardless of whether their host plants were in their native or exotic ranges. Interestingly, both the native and invaded soils hosted diverse local AMF assemblages. Therefore, alien plant invasions were not limited to soils with low AMF diversity. Some AMF taxa could be context-dependent passengers rather than drivers of alien plant invasions. Further studies should identify functions of AMF missing or less abundant in roots of plants growing in exotic ranges.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF, Glomeromycotina) sensu Spatafora et al. (2016) are globally distributed symbionts of plant roots (Smith & Read, 2008). AMF provide crucial support for the establishment of most terrestrial plants, including over 80% of invasive plant species (Cronk & Fuller, 2013). However, many knowledge gaps remain with regard to invasions that do not select for directional shifts in AMF associations (Bunn et al., 2015). While studies comparing AMF of native and alien plants in a single region are common, there is a lack of comparative studies focused on the same species in their native and exotic ranges. The comparison between locally present native and invasive species could be considerably affected by the host identity of AMF as plant species likely support only partially overlapping AMF communities (Husband et al., 2002; Vandenkoornhuyse et al., 2002). Therefore, the comparisons of the AMF of locally present native and invasive species make little sense when searching for changes in the AMF communities that are associated with plant invasion.

In novel environments, the AMF have the potential to alter nutrient acquisition strategies and decrease requirements for the presence of specialized nutrient

TABLE 1 List of examined host plant species, including the indication of their native and exotic ranges

English name	Scientific name	Native range	Exotic range
Species native to North America			
Common ragweed	*Ambrosia artemisiifolia* L.	Eastern parts of North America	Has expanded globally; it has been present in the Czech Republic since 1883
Canadian fleabane	*Conyza canadensis* (L.) Cronquist	North America	Has expanded globally; in the Czech Republic, it has been considered a common species since the first botanical surveys
Annual fleabane	*Erigeron annuus* (L.) Pers.	North America	Europe, East Asia, Oceania and Central America
Canada goldenrod	*Solidago canadensis* L.	Northeastern USA and southern Canada	Eurasia, Australia and New Zealand
Lance-leaved aster	*Symphyotrichum lanceolatum* (Willd.) G.L.Nesom	North America	Europe and New Zealand
Species native to Europe			
Yellow bluestem	*Bothriochloa ischaemum* (L.) Keng	Palearctic	North America
Prickly lettuce	*Lactuca serriola* L.	Palearctic	Introduced globally
Black medick	*Medicago lupulina* L.	Palearctic	Introduced globally
Ribwort plantain	*Plantago lanceolata* L.	Palearctic and Indo-Australian regions	The Americas and South Africa
Common sowthistle	*Sonchus oleraceus* L.	Europe	Introduced globally
White clover	*Trifolium repens* L.	Palearctic	Introduced globally

acquisition strategies of invasive plants (Palma et al., 2017). On the other hand, invasive plants might be less dependent on generalist AMF in their exotic ranges (Seifert et al., 2009), although the reverse has been reported for several species of trees (Moyano et al., 2020; Moyano et al., 2021). However, invasive plants that colonize disturbed areas may obtain, or require, fewer benefits from their AMF partners as long as resources are readily available (Jasper et al., 1989). Generally, there is a lack of quantitative studies that apply a biogeographic perspective to explain exotic plant success (Hierro et al., 2005). However, biogeographic studies have the potential to elucidate how new ecological interactions in the exotic ranges affect the resistance of native biota during the establishment phase of plant invasions (Sun et al., 2015).

We addressed the differences in AMF diversity in 11 mycorrhizal plant species (Table 1), which we examined both in their native and exotic ranges. All 11 species have established dense stands at multiple sites in Europe and North America. Five of the examined host plant species were native to the United States and exotic in Europe, whereas another six species were native to Europe and exotic in the United States (Table 1). We hypothesized that the operational taxonomic unit (OTU)/genus richness and diversity of root-associated AMF of alien plant species in their exotic ranges would be lower than those of conspecifics in their native ranges. The bottleneck effect may apply at the OTU/genus level during the invasion. We further hypothesized that rhizosphere soils in exotic ranges of alien plants have lower OTU/genus richness and diversity of AMF than rhizosphere soils in native ranges of their conspecifics. Therefore, these soils would represent the more degraded environments. We focused on differences and similarities in the OTU and genus richness and diversity, including the overlaps in OTUs/genera among paired root and soil samples and groups of sampling sites in the exotic and native ranges of the plants.

EXPERIMENTAL PROCEDURES

Sampling design

According to the DAISIE database, Europe has 8565 alien invasive plant species (Roy et al., 2019), with at least 5000 alien invasive plant species known from the United States (Pimentel et al., 2005). The present study focuses on plant species common both in their native and exotic ranges, typically within the European and United States study regions. All analysed plant species were abundant in the study regions, and the study regions were not at the edges of their geographic ranges.

We examined the AMF of 11 plant species, including five species that are native to the United States but considered alien and invasive in the Czech Republic and Slovakia (*Ambrosia artemisiifolia*, *Conyza*

canadensis, *Erigeron annuus*, *Solidago canadensis* and *Symphyotrichum lanceolatum*) and another six species that are native to the Czech Republic and Slovakia but considered alien and invasive in the United States (*Bothriochloa ischaemum*, *Lactuca serriola*, *Medicago lupulina*, *Plantago lanceolata*, *Sonchus oleraceus* and *Trifolium repens*) (Table 1). Data on AMF colonization of these plant species are available; however, the methods differed across studies, restricting direct comparisons of data from the United States and Europe for each plant species.

For each species, we examined four sampling sites in the native distribution range and four sites in the invaded range. The examined sites are listed in Table S1; the study regions consisted of the Czech Republic and Slovakia in Europe (48^042^0–50^009^0 N, 13^015^0–21^057^0 E) and Oklahoma in the United States (34^059^0–36^009^0 N, 96^016^0–111^033^0 W). For each plant species, we selected sampling sites that differed in location (see Table S1 for geographic coordinates), differed in plant cover, invasive species abundance, and were of variable invasion duration. The sampling sites varied in size depending on the landscape fragmentation. All sampling sites were separated by at least 10 km distance or by physical barriers. Therefore, sampling sites did not represent identical stands. We sampled in total 78 independent sampling sites, from which we obtained 96 plant/site-specific samples. Therefore, we analysed the AMF of two or more alien species at some sampling sites.

All the tested plants are common in both study regions, and their use in the present study complies with all international, national and/or institutional guidelines. Relevant permits for the collection of study plants were obtained wherever necessary. The collected plants were identified by M. Ř. Voucher specimens of the plants have not been deposited, as the study focused on the associated AMF rather than the plants themselves and as the study focused on common plant species.

Characterization of the sampling sites

We analysed the basic soil characteristics of each selected site in the present study (Table S2). At each sampling site, we collected five samples, each of 200 g of soil. These five samples from a single sampling site were pooled, properly homogenized and sieved through a 2 mm mesh; we used 10 g of each soil sample for subsequent analyses. We incubated the 10 g soil sample with 25 ml of distilled water for 1 h in an orbital shaker, sedimented the sample for another hour, and measured the pH of the supernatant. In addition, we measured the contents of Ca, Mg, K and P using the Mehlich III flame atomic absorption spectroscopy technique. We also quantified the total nitrogen present in the form of NO_3 and NH_4 from samples that were mineralized with sulfuric acid and selenium as catalysts using spectrophotometric detection of the total nitrogen content with a San Plus SKALAR System analyser and the Bertholet reaction.

Processing of root and soil samples

At each sampling site, we collected four individuals of each respective plant species. We placed the collected plants into plastic bags and transferred them to a refrigerator (4^0C). Following the collection of the samples, we washed the roots using cold tap water and processed them for further analyses. We selected undamaged roots with live tissue present in the root cortex for examination.

Concerning soil samples, at each sampling site, we collected a soil sample from the microhabitat of each of the four individuals of each respective plant species. These individual plants were identical to those from which we took the root samples. We defined a microhabitat as soil present in the surroundings of the sampled plant (within a 10 cm diameter surrounding the plant). The soil sample consisted of topsoil collected to a depth of 15 cm. We then homogenized soil samples from each of the four conspecifics within a site. We then dried the soil, sieved through a 2 mm mesh, and processed for further analyses.

DNA extraction

To extract the DNA from roots, we used a NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions using a cetyltrimethylammonium bromide-based PL1 lysis buffer. We estimated the content and quality of the resulting DNA by NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

To extract DNA from soil, we used a DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions with the modifications specified below. Prior to vortexing the soil with the lysis solution, we incubated the samples for 10 min at 70^0C and subsequently vortexed the samples for 15 min.

DNA amplification and sequencing

To amplify the ITS2 rDNA locus, we employed a two-step procedure. In the first step, we amplified DNA using the primers SSUmAf (equimolar mixture of SSUmAf1 and SSUmAf2) and LSUmAr (equimolar mixtures of LSUmAr1 through LSUmAr4) (Krüger et al., 2009). The PCR reaction mix consisted of 5.5 µl of PCR water, 12.5 µl of the Combi PP Master Mix (Top-Bio, Vestec,

Czech Republic) enriched with 0.05 U of the *Pfu* DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA), 2.5 μl of the mixture of forward primers (10 μM each), 2.5 μl of the mixture of reverse primers (10 μM each) and 2 μl of the 10-times diluted DNA extract. We used the following cycle: initial denaturation: 95^{0}C for 5 min, 38 cycles of 95^{0}C for 0.75 min, 58^{0}C for 1.5 min and 72^{0}C for 4.5 min; and final elongation at 72^{0}C for 10 min.

In the second step, we used a set of tagged primers 5.8S (Řezáčová et al., 2019) and ITS4 (White et al., 1990) that amplify 300–400-bp-long DNA regions spanning the highly variable ITS2 rDNA locus, which is suitable for further sequencing on the Illumina platform. The PCR reaction mix consisted of 9.5 μl of PCR water, 12.5 μl of Combi PP Master Mix (Top-Bio), 1.25 μl of the 10 μM forward primers, 1.5 μl of the 10 μM reverse primers and 0.5 μl of the DNA template from the first PCR reaction. We used the following cycle: initial denaturation at 95^{0}C for 5 min, 23 cycles of 95^{0}C for 0.5 min, 58^{0}C for 0.17 min and 72^{0}C for 1 min; and final elongation at 72^{0}C for 10 min.

We checked the quality and quantity of the resulting PCR products using agarose electrophoresis and purified the PCR products using the QIAquick PCR Purification Kit (Qiagen). We then sequenced equimolar amplicon libraries on a 2 x 300-bp MiSeq platform (Illumina, San Diego, CA, USA). We used PCR water as a negative control for the presence of contaminants in the PCR reaction and a sham sampling procedure to ensure the sampling equipment was not contaminated. The sham sampling procedure consisted of using autoclaved sand that had been collected and processed following the same procedures as the experimental samples.

Data analyses

We processed the obtained sequences using SEED 2.0.4 (Větrovský & Baldrian, 2013). The data processing consisted of the assembly of the paired-end reads, removal of the low-quality reads and short reads (<250 bp), and extraction of the ITS2 region using the built-in ITSx 1.0.11 algorithm. We then clustered the ITS2 sequences into OTUs by applying a 97% similarity threshold. We also checked for chimaeras using the built-in USEARCH 8.1.1861 algorithm. We performed NCBI nucleotide BLAST searches to classify the identified OTUs into species, and pooled the sequences that were affiliated with the same OTU. To analyse changes at the genus level, we pooled the sequences according to the AMF genera. We identified the genera based on the NCBI Blast analysis of the obtained ITS2 sequences. For unknown genera, we indicated the name of the identifiable higher taxon. This could result in a slight underestimation of the total number of genera. However, it is not a complication for comparisons of relative abundances among the samples that were all processed in the same way. We excluded the non-Glomeromycotinan sequences from further analyses. We further rarefied the remaining sequences to retain 2400 Glomeromycotina sequences per sample. Samples that yielded <2400 sequences were retained in the dataset because the lower numbers of sequences did not affect the calculation of relative ratios when comparing the native and invaded populations of host plants.

In total, we identified 6,832,842 sequences (reads), from which we excluded 656,349 sequences that represented chimeric clusters. We grouped the obtained sequences into 115,776 clusters, of which we considered 9445 to represent chimaeras. We used the remaining 106,331 clusters in subsequent analyses. This number was further decreased to 101,431 clusters after removing non-Glomeromycotina sequences, non-ITS2 sequences and complementary strand sequences, and short sequences.

The average partitioning of the clusters among the AMF, no hits and organisms other than AMF was 87.6%, 9.9% and 2.5%, respectively. Note that these clusters were identified based on 97% sequence identity. Therefore, any currently known ~3670 Glomeromycotina species (according to the UNITE database of ITS sequences) could be represented by multiple related clusters.

We subsequently analysed the diversity of the detected OTUs and genera using a panel of biodiversity indices, including the number of observed taxa, number of shared taxa, Sørensen index, and Chao–Sørensen raw abundance-based index, Morisita–Horn index and Bray–Curtis index. We calculated Dominance as 1—Simpson index, where 1 indicates complete domination of a single taxon and 0 indicates the equal representation of all taxa. Fisher's alpha is a parametric diversity measure assuming the abundance of a particular taxon follows the log series distribution. Equitability is an evenness measure, where the Shannon index is divided by the logarithm of the number of document types; the Shannon index is a measure of entropy, ranging from 0 for datasets containing a single taxon to high values for datasets with many taxons. The Berger–Parker dominance index is calculated as the number of individuals in the dominant taxon relative to the total number of individuals (Harper, 1999). We calculated the number of shared taxa (shared OTUs and shared genera), which represented the number of taxa that were shared between samples from native and exotic ranges of the respective plant or between the root-associated AMF and soil-dwelling AMF that were sampled from the identical microhabitat. We employed a canonical correspondence analysis to determine the contributions of multiple variables to the variation in the data, followed by the non-metric

multidimensional scaling analysis (Gower similarity measure) of AMF genera to analyse the effects of explanatory variables. We used paired *t*-tests to analyse differences in diversity indices between the native and invaded habitats. We performed all calculations in SigmaPlot 12.0, EstimateS 9.1.0 and PAST 2.14. Data are shown as the mean ± SD unless stated otherwise.

RESULTS

Root-associated AMF assemblages of plants in their native and invaded distribution ranges

The analysed assemblages of AMF in roots differed strongly in their shared OTUs and the corresponding diversity indices when comparing samples from the native and invaded distribution ranges of respective conspecifics. The number of shared OTUs ranged from 6.4% to 44.3% of the total OTUs found in the exotic range of the respective plant (Figure S1).

The number of AMF genera was higher at native sites (paired *t*-test $p = 0.03$), which also hosted assemblages with lower dominance (paired *t*-test $p = 0.02$). The entropy and alpha diversity were similar among the paired root samples (paired *t*-test $p > 0.05$ for both variables) (Figure S2). The values of the Berger–Parker index were similar at the native and invaded sites or higher at the invaded sites (paired *t*-test $p = 0.03$) (Figure S2).

The genera *Glomus*, *Paraglomus* and *Pervetustus* dominated the assemblages. The Czech samples (but not the United States samples) of *T. repens* (native range) and *M. lupulina* (exotic range) were colonized by *Acaulospora*, *Archaeospora*, *Claroideoglomus* and *Diversispora*. Colonization of *E. annuus* also differed between the two regions. In the United States (native) range, it was abundantly colonized by *Acaulospora*, Ambisporaceae gen. sp., *Dominikia* and *Gigaspora*. In contrast, in its exotic range, these taxa were nearly absent from the analysed roots of *E. annuus*. They were replaced by diverse but not overly abundant representatives of *Archaeospora*, *Cetraspora*, *Claroideoglomus*, *Diversispora* and *Funneliformis* (Table S3).

Soil-dwelling AMF assemblages in native and invaded habitats

The number of soil-associated AMF OTUs did not correlate with the invasion status of the examined plants (Figure S3). All analysed assemblages were characterized by low dominance. The number of OTUs shared between native and exotic ranges of paired conspecifics ranged from 12 to 47, which corresponded to low values of the classic Jaccard and Sørensen indices (Jaccard ≤0.12 and Sørensen ≤0.22) (Figure S3). However, when analysed at the level of AMF genera, their richness and diversity were similar in soils from the native and invaded habitats (Figure S4).

Analysed AMF assemblages in the soil samples were dominated by *Glomus*. Two additional genera, *Paraglomus* and *Pervetustus*, were dominant in the root samples but had much lower relative abundance in the soil samples. Interestingly, while the differences in *T. repens* and *M. lupulina* colonization by AMF were consistent with the AMF diversity observed in the corresponding soil samples, the differences in AMF of *E. annuus* were independent of the spectrum of AMF found in the corresponding soil samples (Table S3).

Comparison of OTUs and genera of AMF in the corresponding root and soil samples

The analysed AMF assemblages contained only a limited number of OTUs that were shared between the plant root samples and soil samples from microhabitats of the same plant species (Figure 1). We found the highest values of the classic Jaccard and Sørensen indices when comparing root and soil samples in exotic ranges of *A. artemisiifolia* and *S. lanceolatum*, and in native habitats of *T. repens* and *P. lanceolata* (Figure 1).

The number of shared AMF genera was higher in the native ranges than in the exotic ranges; however, they were identical for *A. artemisiifolia* and lower at the native sites for *E. annuus* (13 vs. 19 genera) and *S. lanceolatum* (18 vs. 19 genera) (Figure S5). The values of the classic Jaccard and Sørensen indices were generally high in both native and exotic ranges (Figure S5).

Root-associated AMF assemblages are more diverse

The largest part of the variability of root-associated AMF was related to the native/invaded habitat status, whereas the plant species had less of an effect [Figure 2(A)]. The AMF genera were distributed unevenly with respect to the sampling region and in the native/invaded habitats (Figure S6). The obtained values varied strongly according to the individual host plant species, without any shared patterns (Figure S6). In contrast, soil-dwelling AMF assemblages had opposite relationships between samples from the native and invaded habitats [Figure 2(B)]. The native/invaded habitat gradient was associated with the differences between paired root-associated and soil-dwelling AMF assemblages, while the plant species-derived variability was less pronounced (Figure S7).

Country	US	CZ	CZ	CZ	CZ	US	CZ	US	US	US	US
...Native pair	CZ	US	US	US	US	CZ	US	CZ	CZ	CZ	CZ
Status	invade	invad	invad	invad	invad	invad	invad	invad	invad	invad	invad
...Native pair	native	native	native	native	native	native	native	native	native	native	native
Species acronym	SonO	ConC	SolC	AmbA	EriA	BotI	SymL	MedL	LacS	TriR	PlaL
Sobs First Sample	169	259	483	324	509	37	494	248	230	194	104
...Native pair	195	120	207	210	129	225	295	466	339	531	523
Sobs Second Sample	73	148	194	235	182	156	293	260	110	159	160
...Native pair	202	123	123	152	145	204	138	220	161	253	303
Shared Species Observed	35	67	132	127	136	11	201	115	54	58	42
...Native pair	32	28	54	62	49	82	85	144	106	181	213
Jaccard Classic	0.169	0.2	0.24	0.29	0.25	0.06	0.34	0.29	0.19	0.2	0.19
...Native pair	0.088	0.13	0.2	0.21	0.22	0.24	0.24	0.27	0.27	0.3	0.35
Sorensen Classic	0.289	0.33	0.39	0.45	0.39	0.11	0.51	0.45	0.32	0.33	0.32
...Native pair	0.161	0.23	0.33	0.34	0.36	0.38	0.39	0.42	0.42	0.46	0.52
Chao–Sorensen-Raw Abundance-based	0.759	0.35	0.67	0.68	0.6	0.29	0.78	0.74	0.47	0.57	0.51
...Native pair	0.448	0.28	0.45	0.54	0.58	0.73	0.67	0.57	0.63	0.76	0.85
Morisita–Horn	0.616	0.2	0.47	0.29	0.44	0.11	0.38	0.5	0.29	0.22	0.14
...Native pair	0.184	0.13	0.36	0.13	0.41	0.32	0.16	0.06	0.19	0.76	0.31
Bray–Curtis	0.316	0.12	0.27	0.22	0.21	0.08	0.25	0.2	0.17	0.15	0.11
...Native pair	0.104	0.05	0.17	0.13	0.21	0.21	0.14	0.13	0.11	0.22	0.25

FIGURE 1 Comparison of OTU richness and diversity of AMF in matched samples from roots and corresponding soil samples in both native and invaded distribution ranges of the examined host plants. The colour code is set individually for each parameter; red indicates high values and blue indicates low values. The species abbreviations are as follows: BotI, *Bothriochloa ischaemum*; ConC, *Conyza canadensis*; EriA, *Erigeron annuus*; PlaL, *Plantago lanceolata*; SolC, *Solidago canadensis*; LacS, *Lactuca serriola*; TriR, *Trifolium repens*; SymL, *Symphyotrichum lanceolatum*; AmbA, *Ambrosia artemisiifolia*; SonO, *Sonchus oleraceus*; MedL, *Medicago lupulina*. 'Country' indicates the origin of samples from invaded distribution ranges; 'second sample' indicates the origin of samples from native ranges. The list of sampling sites from where the samples were collected is provided in Table S1. OTU, operational taxonomic unit; AMF, arbuscular mycorrhizal fungi

DISCUSSION

The present study identified a clear distinction between the AMF assemblages associated with the 11 selected plant species from their native and exotic ranges (Figures S1, S4 and S6). However, compared to native stands of conspecifics, AMF assemblages in exotic ranges were not necessarily associated with lower OTU richness. Instead, a slight decrease in the number of genera was present. This difference was restricted to root-associated samples and was absent from the associated soil samples. When the detected OTUs were clustered according to the genera of the AMF, the soil samples from the native and invaded sites did not show any shared pattern. In contrast, when AMF assemblages from root samples of the same sites were analysed, native stands hosted more fungal genera than invasive conspecifics. Therefore, plants growing in invaded habitats were associated with simplified AMF assemblages, which lacked some AMF species that were common in the native habitats but retained the overall OTU richness. Further research should address the contributions of the individual AMF taxa to the plant–soil feedback theory sensu Bever et al. (2012). The plant–soil feedback theory suggests that the invasion success of exotic plants could be mediated by greater AMF richness, a greater abundance of specific AMF taxa, or a greater ability to colonize the host among specific AMF taxa in invaded habitats (Klironomos, 2002; Reinhart et al., 2003; van Grunsven et al., 2007; Westover & Bever, 2001).

We found all root-associated AMF assemblages to be OTU-rich. However, the assemblages of the 11 analysed plants differed strongly in the numbers of OTUs shared between their corresponding native and invaded sites. The finding of OTU-rich assemblages was surprising given that previous molecular studies have reported relatively low numbers of OTUs associated with the invasive species that were analysed in the present study (Betekhtina et al., 2016; Hazard et al., 2013; Jia et al., 2018a; Jia et al., 2018b; Reininger et al., 2015; Yuan et al., 2014; Zhang et al., 2018).

Previous research on the AMF of each study species is summarized in Table S4. Generally, the previous studies identified much lower AMF diversity to be associated with the study host species. This applied both to the native and invaded ranges. The number of previously reported species or OTUs was often lower than the number of genera found in the present study (Table S4) (Betekhtina et al., 2016; Hazard et al., 2013; Jia et al., 2018a; Jia et al., 2018b; Johnson et al., 2003; Majewska et al., 2015; Reininger et al., 2015; Renker et al., 2005; Shah et al., 2015; Wilson & Hartnett, 1998;

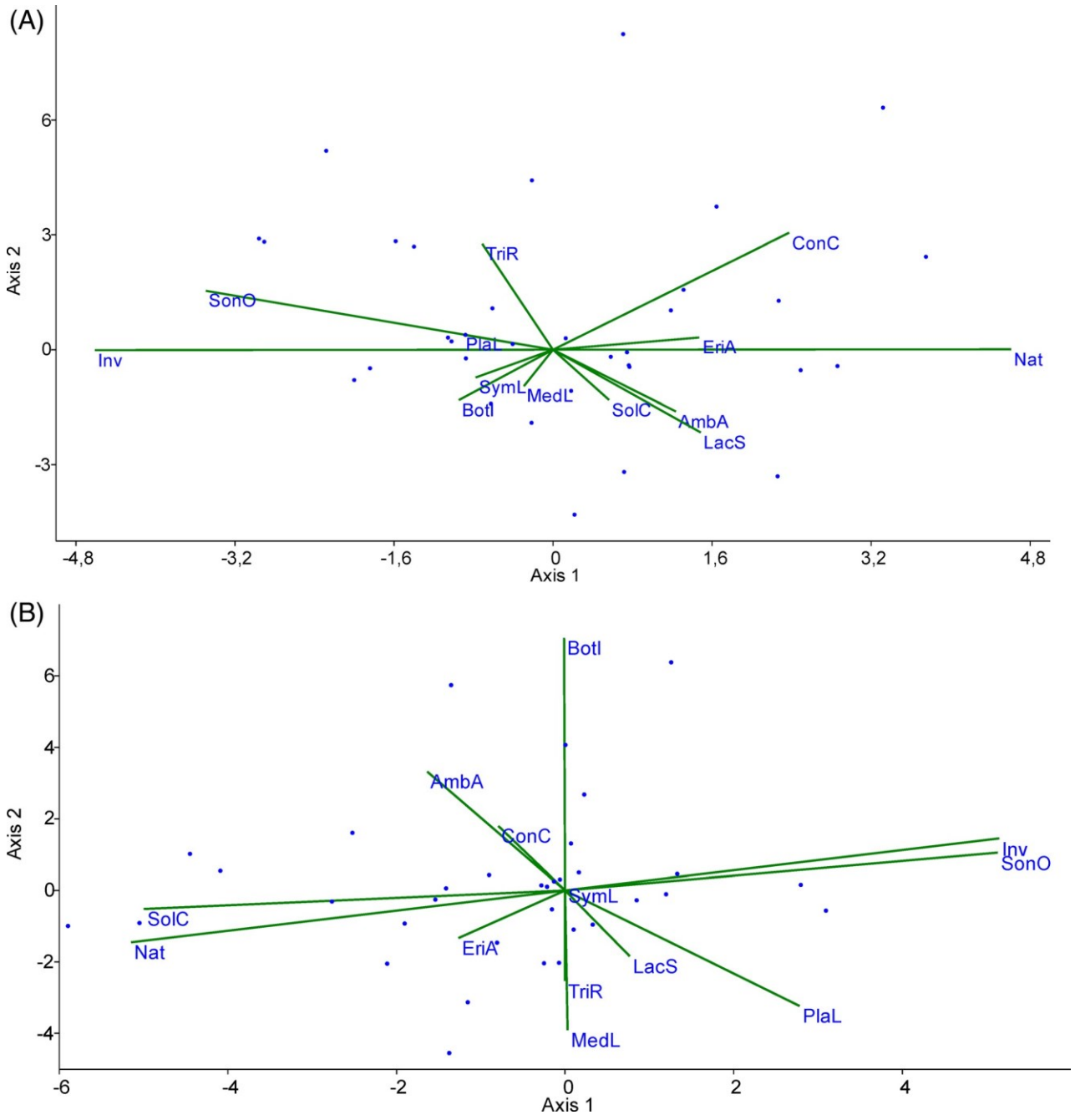

FIGURE 2 Canonical correspondence analyses of the AMF sampled from roots (A) and corresponding soils (B). (A) Axis 1 eigenvalue: 0.12, 33.4% of the variability explained; axis 2 eigenvalue: 0.05, 15.3% of the variability explained. (B) Axis 1 eigenvalue: 0.15, 26.0% of the variability explained; axis 2 eigenvalue: 0.09, 15.8% of the variability explained. The species abbreviations are as follows: BotI, *Bothriochloa ischaemum*; ConC, *Conyza canadensis*; EriA, *Erigeron annuus*; PlaL, *Plantago lanceolata*; SolC, *Solidago canadensis*; LacS, *Lactuca serriola*; TriR, *Trifolium repens*; SymL, *Symphyotrichum lanceolatum*; AmbA, *Ambrosia artemisiifolia*; SonO, *Sonchus oleraceus*; MedL, *Medicago lupulina*. 'Inv' indicates samples collected in exotic ranges, 'Nat' indicates samples collected in native ranges. AMF, arbuscular mycorrhizal fungi

Yuan et al., 2014; Zhang et al., 2018; Zhang et al., 2020).

Similar to the study by Nelson and Karp (2013) assessing native and invaded *Phragmites australis* populations, we found the assemblages identified in the present study differed not only in AMF OTU composition but also in the relative abundances of specific taxa. Whether the OTUs with increased dominance at invasive sites contribute to the performance and competitiveness of the respective host plant remains to be tested. Moreover, there is an unresolved dilemma regarding whether specialized AMF are central to the establishment of particular invasive plant species or if generalist AMF species with a broad host range play a

role. Both options may apply to specific plants and environments. The native and invasive populations of the plants were associated with distinct assemblages of AMF, which may reflect different potential mechanisms of plant growth facilitation in native and exotic ranges. The management implications are specific for individual host species. Native populations of some species, particularly *P. lanceolata*, *T. repens* and *L. serriola*, had a high similarity of root-associated AMF OTUs with those found in the surrounding soils and their assemblages of root-associated AMF at native sites were OTU-rich. However, the same species displayed much lower OTU richness at invaded sites, where they also shared substantially fewer OTUs with the surrounding soils. Some of the genera of the AMF were much less abundant in one of the two analysed regions; we observed this pattern, for example, for root-associated *Rhizoglomus*, *Oehlia* and *Archaeospora* spp. The genera *Dominikia*, *Kamienskia* and *Microdominikia* were much more common in the United States-based samples from both native and invaded stands. Comparing native and invaded stands, the roots of plants in invaded stands hosted more *Acaulospora*, Ambisporaceae gen. sp., *Cetraspora*, Diversisporaceae gen. sp., *Gigaspora* and *Kamienskia* spp. For some genera, such as *Acaulospora*, *Cetraspora*, or *Kamienskia*, these differences were restricted to root-associated AMF only, as they were equally present in soils that were collected in the native and exotic ranges of the plants. Only *Septoglomus* was present more frequently in root samples from its exotic ranges (and was equally present in soil samples from both ranges); no other genera were more abundant in the root and soil samples from the exotic ranges than in the samples from the native ranges.

The present study has several limitations. First, as it had an observational design, its conclusions should be verified by an appropriately defined experimental study. Second, the conclusions were based on DNA analyses, and further research should also corroborate these data with microscopical examinations, particularly to obtain insights into AMF physiology by scoring fungal structures (Bodenhausen et al., 2021). The next-generation sequencing is a method that cannot be used to generate absolute quantities due to secondary structure or GC content of the resulting amplicons, generation of false diversity from sequencing error or chimaera formation, primer mismatches, and the presence of multiple copies of the ITS2 locus in some species. However, as any such bias affects equally all analysed samples, the obtained data offer a semi-quantitative approximation of the OTU relative abundance, allowing direct semi-quantitative comparison of the analysed samples (Ong et al., 2013; Wang et al., 2013).

In conclusion, during the expansion of the 11 selected plant species from Europe relocated to the United States and from the United States to Europe, some AMF taxa remained associated with the roots of the respective host plant species. An important management implication stems from the fact that although some fungal taxa were more abundant in the examined microhabitats on one of the continents, these taxa failed to colonize the exotic plant species. Instead, the root-associated AMF assemblages were simplified, which manifested as the loss of several genera in the exotic ranges of the plants. The examined root-associated assemblages of AMF also faced increased dominance, which is likely tightly linked to the simplification of the structure of fungal assemblages. The dominant fungal genera were generally present regardless of whether their host plants were in their native or exotic ranges. Some AMF could be context-dependent passengers rather than drivers of alien plant invasions, and controlled laboratory studies are needed to identify functions of the AMF found to be missing or less abundant in roots of plants growing in their exotic ranges. The obtained data suggest that despite the process of invasion being associated with the simplification of root-associated AMF assemblages in exotic ranges, the host species managed to acquire some of the local OTUs. In three plant species, the numbers of OTUs shared between the soil and root samples were higher in their exotic ranges compared to their native ranges.

AUTHOR CONTRIBUTIONS

V.Ř., G.W.T.W., M.Ř. and E.B.D. conceived and designed the experiments, V.Ř., M.Ř. and E.B.D. harvested the samples, V.Ř., M.Ř., E.B.D., T.M. and P.H. performed the analyses, M.G. and P.H. analysed the data, and P.H. wrote the paper, V.Ř. is responsible for the integrity of this work. All authors revised the article's intellectual content and approved the final version.

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CONFLICT OF INTEREST

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DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this published article and its supplementary information files.

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