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RESEARCH ARTICLE

Sympatric diploid and tetraploid cytotypes of *Centaurea stoebe* s.l. do not differ in arbuscular mycorrhizal communities and mycorrhizal growth response

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PREMISE OF THE STUDY: Genome duplication is associated with multiple changes at different levels, including interactions with pollinators and herbivores. Yet little is known whether polyploidy may also shape belowground interactions.

METHODS: To elucidate potential ploidy-specific interactions with arbuscular mycorrhizal fungi (AMF), we compared mycorrhizal colonization and assembly of AMF communities in roots of diploid and tetraploid *Centaurea stoebe* s.l. (Asteraceae) co-occurring in a Central European population. In a follow-up greenhouse experiment, we tested inter-cytotype differences in mycorrhizal growth response by combining ploidy, substrate, and inoculation with native AMF in a full-factorial design.

KEY RESULTS: All sampled plants were highly colonized by AMF, with the Glomeraceae predominating. AMF-community composition revealed by 454-pyrosequencing reflected the spatial distribution of the hosts, but not their ploidy level or soil characteristics. In the greenhouse experiment, the tetraploids produced more shoot biomass than the diploids did when grown in a more fertile substrate, while no inter-cytotype differences were found in a less fertile substrate. AMF inoculation significantly reduced plant growth and improved P uptake, but its effects did not differ between the cytotypes.

CONCLUSIONS: The results do not support our hypotheses that the cytotype structure in a mixed-ploidy population of *C. stoebe* is mirrored in AMF-community composition and that ploidy-specific fungal communities contribute to cytotype co-existence. Causes and implications of the observed negative growth response to AMF are discussed.

KEY WORDS 454-sequencing; arbuscular mycorrhizal fungi; *Centaurea maculosa*; co-existing cytotypes; inter-cytotype differences; mixed-ploidy population; mycorrhizal symbiosis; ploidy level; spotted knapweed.

Polyploidy, i.e., whole-genome duplication, is widespread in flowering plants and is associated with profound and multi-faceted changes at the cytological, morphological, physiological, developmental and functional levels (Soltis et al., 2010). Phenotypic and ecological consequences of genome duplication, such as altered growth and phenology, secondary metabolite production, and habitat preferences may influence not only interactions with other species within plant communities, but also interactions with other trophic levels (Ramsey and Ramsey, 2014). There is compelling evidence for inter-cytotype differences in the frequency of pollinator visits and/or herbivore attack as well as in the composition of associated insect communities (e.g., Kennedy et al., 2006; Halverson et al., 2008; Thompson and Merg, 2008; Münzbergová et al., 2015). In contrast, little is yet known on how differences in plant ploidy level affect belowground interactions with symbiotic fungi. This gap is surprising given that the vast majority of vascular plants enter into partnership with some type of mycorrhizal symbiosis, most frequently with arbuscular mycorrhizal fungi (AMF; Smith and Read, 2010).

Obligatory biotrophic AMF from the subphylum Glomeromycotina (Spatafora et al., 2016) are tightly coupled with their hosts because they completely depend on plant-derived carbon in

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exchange for improving acquisition of less mobile nutrients (especially phosphorus) and enhancing tolerance to biotic and abiotic stress (Smith and Read, 2010). Due to these reciprocal services, naturally omnipresent AMF can modulate host plant growth and play an important role in the functioning of plant communities and ecosystems (van der Heijden et al., 1998; Vogelsang et al., 2006; Lin et al., 2015). Depending on the balance between mycorrhizal benefits and costs, the effects of AMF on host growth (i.e., mycorrhizal phenotype) range from beneficial to deleterious (Johnson et al., 1997) and are highly dependent on the abiotic and biotic context (Hoeksema et al., 2010). Less mutualistic (or even parasitic) mycorrhizal associations have been reported, e.g., for abiotic conditions reducing mycorrhizal benefits such as high phosphorus availability and nitrogen limitation or for conditions increasing mycorrhizal costs such as long-term shading leading to carbon limitation (Johnson et al., 1997, 2015).

In addition to the abiotic context, the mycorrhizal phenotype also depends on the identity of both plant and fungal partners (Johnson et al., 2015). Different combinations of host plants and AMF were shown to yield different results in terms of both plant growth and performance of AMF (e.g., Helgason et al., 2002; Klironomos, 2003). A high functional diversity of arbuscular mycorrhizal (AM) symbiosis has been observed not only at the level of different fungal/plant species, but even among isolates of the same AMF species on one side (e.g., Munkvold et al., 2004; Ehinger et al., 2012; Mensah et al., 2015) and among different cultivars or populations of the same plant species on the other side (e.g., Hetrick et al., 1993; Doubková et al., 2012; Taylor et al., 2015). Taking into account that both ploidy level and symbiosis with AMF influence traits related to plant fitness and stress tolerance, we hypothesize that there is ploidy specificity in plant–AMF interactions (Sudová et al., 2010, 2014). The polyploids have increased demand for nutrients needed to synthesize the additional DNA (Leitch and Leitch, 2008; Šmarda et al., 2013), which is by mass approximately 39% nitrogen and nearly 9% phosphorus (Sterner and Elser, 2002). Therefore, the polyploids were suggested to benefit more from symbiosis with AMF than diploids particularly under nutritional limitation (Segraves and Anneberg, 2016).

Due to the contrast between a low number of known AMF species and a much higher diversity of host plant species, AM symbiosis has long been regarded as nonspecific (Smith and Read, 2010). However, molecular tools revealed that the actual diversity of Glomeromycotina could be considerably higher than expected (Öpik et al., 2014). In addition, there is increasing evidence that AMF communities in roots of co-existing plant species or even cultivars are distributed nonrandomly (e.g., Vandenkoornhuyse et al., 2003; Sýkorová et al., 2007; Davison et al., 2011). Host identity therefore seems to exert a selective pressure, of varying degree, on the structure of AMF communities. It has been shown that mycorrhizal plants are able to preferentially support fungal species that provide them with more nutrients (Bever et al., 2009; Kiers et al., 2011) and vice versa, that fungal partners are also able to reflect host plant quality (C status) and preferentially allocate nutrients to high-quality hosts (Hammer et al., 2011; Kiers et al., 2011; Fellbaum et al., 2014).

Arbuscular mycorrhizal fungi are known to strongly differ in their physiological and growth traits (e.g., carbon requirements, phosphorus translocation efficiency, or investment into extraradical/intraradical biomass) and provide their hosts with different services (Hart and Reader, 2002; Maherali and Klironomos, 2007; Chagnon et al., 2013). Considering inter-cytotype differences in nutrient demands and habitat preferences (Levin, 2002), we hypothesize that co-existing host plant cytotypes may be colonized by different AMF that specifically fulfill their requirements and by this contribute to cytotype coexistence. In a recent comprehensive review on how host plant polyploidy may affect the communities of associated organisms, Segraves (2017) posited that decreased photosynthetic rate of polyploids may shift AMF species composition to those requiring less carbon and that increased requirements of polyploids for phosphorus and nitrogen may lead to the promotion of AMF species more effective in P foraging and to the loss of species with high N requirements.

Here, we address potential inter-cytotype differences in association with AMF in a mixed-ploidy population of spotted knapweed (*Centaurea stoebe* s.l.). Using cytotypes that occur sympatrically (further referred to as "co-existing cytotypes") allows us to reduce potential confounding effects of larger-scale spatial heterogeneity, dispersal limitation, and local adaptations on structuring and functioning of AMF communities associated with different cytotypes. Although our model species has gained considerable attention in mycorrhizal research in the context of the invasiveness of the tetraploid cytotype in the introduced range (e.g., Callaway et al., 2004a, b; Mummey and Rillig, 2006; Harner et al., 2010; Emery and Rudgers, 2012), to the best of our knowledge, no study has addressed potential interactions of the co-existing cytotypes with AMF. The earlier observations that co-existing *C. stoebe* cytotypes differ at the study site in their microhabitat preferences and colonization abilities led us to hypothesize that the cytotypes might differ in their spectra of mycobionts in their roots and in their growth benefit by mycorrhization. Specifically, we ask the following questions: (1) Are roots of co-existing diploid and tetraploid plants colonized by different communities of AMF? (2) Do diploid and tetraploid individuals respond differently to inoculation with native AMF? To answer these questions, we determined AMF colonization and structure of AMF communities in the roots of field-collected diploid and tetraploid cytotypes of *Centaurea stoebe* s.l. (using 454-pyrosequencing), and compared the growth response of both cytotypes to the inoculation with native AMF in the greenhouse experiment.

MATERIAL AND METHODS

Study species

Centaurea stoebe L. (Asteraceae, syn. *Centaurea maculosa* Lam.) is a herbaceous species distributed from western Asia to western Europe, with the center of the distribution in southeastern and eastern Europe (Meusel and Jäger, 1992; Ochsmann, 2000). It encompasses two cytotypes, diploid (*C. stoebe* s.s., $2n = 2x = 18$) and allotetraploid (*C. stoebe* s.l., $2n = 4x = 36$), which originated by hybridization between the diploid counterpart and a yet unknown but closely related taxon (Mráz et al., 2012a). Both cytotypes are treated as separate taxa, usually at the subspecies level, but their nomenclature remains unresolved (Mráz et al., 2011). Single-cytotype populations clearly prevail in the native range although a few mixed-ploidy populations have been reported (Španiel et al., 2008; Treier et al., 2009; Mráz et al., 2012b). From the introduced range of the species in North America, only tetraploids have been recorded despite the fact that the diploid cytotype is more common in the native range (Müller-Schärer et al., 2004; Mráz et al., 2011). Superior colonization ability of tetraploids has been recorded also in the native range, especially in Central Europe where

tetraploids spread at predominantly anthropogenic sites (Mráz et al., 2012b; Otisková et al., 2014; Rosche et al., 2016). While diploids are annual or biennial monocarpic plants, tetraploids are short-lived perennial polycarpics, often flowering in the first year (Boggs and Story, 1987; Ochsmann, 2000; Müller-Schärer et al., 2004; Mráz et al., 2011; Hahn et al., 2012).

Study site

The interactions of the two cytotypes of *C. stoebe* s.l. with AMF were studied in a mixed-ploidy population at Sandberg hill (Mráz et al., 2012b) in the city of Bratislava, district of Devínska Nová Ves, southwestern Slovakia (48.201 N, 16.974 E, 192–196 m a.s.l.). The study site is covered by thermophilous steppe vegetation growing on Tertiary sands (Feráková and Kocianová, 1997). The site was heavily grazed until the 1950s, but the grazing was completely abandoned in 1964 when the site was declared a Natural Reserve. Importantly, the site was exploited for sand from 1897 until the 1960s (Klačka and Pokorný, 1995). These human-induced disturbances were probably the main cause for the introduction and spread of the tetraploid cytotype into the previously established diploid population and for significant microspatial segregation of the two cytotypes at the scale of centimeters and meters (Appendix S1, see Supplemental Data with this article). Importantly, this patchy cytotype distribution of the sites preferentially occupied either by diploid or tetraploid plants, hereafter called microsites, is strongly correlated with habitat differences. Whereas diploids are more common on undisturbed ("natural") habitats covered by denser vegetation, tetraploids prefer open and more disturbed habitats (Mráz et al., 2012b).

AMF in field-sampled *C. stoebe* s.l. roots

Plant sampling—Arbuscular mycorrhizal colonization in roots of both *C. stoebe* cytotypes was confirmed by preliminary sampling in April 2011 at the rosette stage (data not shown). To determine the precise level of mycorrhizal root colonization (the percentage of root length colonized by AMF) and the composition of AMF communities in roots of both cytotypes, we sampled 15 plants per cytotype at the flowering stage in August 2011. Plant and soil samples were collected across the study site to encompass the spatial distribution of the two cytotypes and include spatially close and distant plants belonging to both cytotypes (cf. Appendix S1). This sampling strategy captures the small (centimeters to several meters) and large spatial scale (several tens of meters) variations in AMF communities between and within the cytotypes at the study site. Entire flowering plants, including their roots, were carefully dug up, transferred with ca. 1 L of soil in plastic bags to the laboratory and stored at 4°C until processed (within 4 d of sampling). Initial plant assignment to ploidy level was based on life history and morphological traits (cf. Mráz et al., 2011). Specifically, both cytotypes can be reliably distinguished by the absence (diploids) or presence (tetraploids) of accessory rosettes, which are clearly visible during flowering and strongly correlated with the differences in the life cycle. Furthermore, we used differences in size and shape of capitula as additional discriminative characters (for further details, see Mráz et al., 2011). Flow cytometric analyses (Suda et al., 2007) confirmed cytotype determinations based on morphology.

Sample processing—Rhizosphere soil was carefully separated from the roots of the sampled plants by manual shaking, thoroughly mixed, dried and sieved (mesh size <2 mm). A subsample of the soil from each plant was then analyzed for pH, cation exchange capacity, organic C, and macronutrients P, N, K, Ca, and Mg (Table 1A; Appendix S2). Total and organic C and N were determined by a combustion method on an NC2500 elemental analyzer (Carlo Erba Instruments, Milan, Italy). Phosphorus was extracted using 0.5 M sodium bicarbonate (Olsen-P) and measured using a Unicam UV-400 spectrometer (ATI Unicam, Cambridge, UK). The available concentrations of K, Ca, and Mg were extracted with 1 M ammonium acetate and measured using a high-resolution continuum source atomic absorption spectrometer ContrAA 700 (Analytik Jena AG, Jena, Germany).

The roots were washed to remove soil particles, dried with a paper towel, and separated into two parts. A small subsample (~100 mg fresh mass) was taken from different parts of the root system of each plant and stained with 0.05% w/v trypan blue in lactoglycerol (Koske and Gemma, 1989). Stained root segments were mounted on slides to assess mycorrhizal root colonization with a compound microscope at 100× magnification using the magnified intersection method (McGonigle et al., 1990). We scored 100 intersections from each root system and recorded the presence of arbuscules, vesicles, and AMF hyphae. In addition, we recorded the presence of microsclerotia and melanized hyphae, that are the typical structures of dark septate endophytes (DSE). Two samples (~100 mg fresh mass) obtained from the other part of the root system were stored at −80°C until used for downstream DNA analyses (except for one tetraploid plant with root volume too small to be used for both mycorrhizal colonization and molecular analyses).

TABLE 1. Characteristics of rhizosphere soil of field-sampled diploid (2*x*) and tetraploid (4*x*) individuals of *Centaurea stoebe* s.l. (A) and homogenized substrates collected from 2*x* and 4*x* microsites and used in the greenhouse experiment (B). For field sampling (A), the data are means (± SE) of 15 independent replicates; significant differences between both substrates according to a one-way ANOVA are marked by different letters. For the greenhouse experiment (B), the data represent means of three subsamples of each of the pooled substrates; therefore, the error values represent measurement errors, and consequently no statistical test was performed.

	(A) Field sampling		(B) Greenhouse experiment	
Variable	2*x*	4*x*	2*x*	4*x*
pH_{H2O}	7.29 ± 0.09[a]	7.56 ± 0.11[a]	8.14 ± 0.04	8.16 ± 0.03
CEC ($mmol_c$ kg^{-1})	99.34 ± 18.35[a]	61.52 ± 6.42[a]	116.27 ± 1.19	48.79 ± 0.71
C_{org} (%)	1.03 ± 0.27[a]	0.47 ± 0.08[b]	0.61 ± 0.04	0.19 ± 0.01
N (%)	0.126 ± 0.032[a]	0.049 ± 0.008[b]	0.099 ± 0.001	0.045 ± 0.001
P (mg kg^{-1})	11.70 ± 1.18[a]	9.09 ± 0.76[a]	11.33 ± 0.26	7.25 ± 0.46
K (mg kg^{-1})	104.22 ± 14.10[a]	76.85 ± 15.66[a]	42.10 ± 0.46	26.05 ± 0.14
Ca (mg kg^{-1})	1769.3 ± 327.5[a]	1110.3 ± 130.9[a]	2211.7 ± 23.2	920.3 ± 15.6
Mg (mg kg^{-1})	96.37 ± 23.98[a]	45.51 ± 4.16[b]	59.32 ± 0.72	25.64 ± 0.79

Molecular methods—The frozen root samples (two replicates per plant) were homogenized using the Tissue Lyser II (Qiagen, Hilden, Germany). DNA was extracted using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions, with a final elution with 50 μL of AE Buffer. Isolated DNA was diluted 10 times with ddH_2O and used as a template for subsequent PCR reactions. We ran two independent PCR reactions from each root sample to avoid PCR bias (i.e., products of four independent PCR reactions were obtained per individual plant). In the first step, AMF-specific primers SSUmAf-LSUmAr were used to amplify partial small subunit (SSU), whole ITS and partial large subunit (LSU) of rDNA (Krüger et al., 2009). The mix for the first PCR reaction included 1× Taq buffer with KCl/without $MgCl_2$, 0.2 mM of each of dNTPs, 2 mM $MgCl_2$, 0.5 μM of each primer, 40 μg of BSA, 2.4 U of DNA polymerase (Pfu DNA polymerase (2.5 U/μL): Taq DNA polymerase (5 U/μL, 1:24) and 5 μL of the template in a total volume of 50 μL. All reagents were from Thermo Scientific (Waltham, MA, USA). Thermal cycling was done in an Eppendorf Mastercycler Gradient (Eppendorf, Hamburg, Germany) with the following conditions: 5 min at 95°C; 38 cycles of 30 s at 95°C, 90 s at 60°C, and 2 min at 72°C; 10 min at 72°C. Four PCR products per individual plant were pooled and purified using the QIAquick PCR Purification Kit (Qiagen) and eluted into 30 μL of ddH_2O. The obtained product was again purified using the Zymoclean Gel DNA Recovery Kit (Zymo Research, Orange, CA, USA) to eliminate short DNA fragments and eluted into 10 μL of ddH_2O. The resulting PCR products were used as a template for the second PCR reaction using tagged eukaryotic primers ITS1-ITS4 (White et al., 1990) to obtain amplicon libraries for 454-pyrosequencing as described by Baldrian et al. (2012). The reaction mix included 1× Phusion HF buffer with 1.5 mM $MgCl_2$, 1.5 μL of DMSO PCR Reagent (Sigma Aldrich, St. Louis, MO, USA), 0.2 mM of each dNTP, 0.1 μM of each tagged primer, 1 U Phusion polymerase (New England BioLabs, Ipswich, MA, USA), and 4 μL of the template in a total volume of 50 μL. Cycling conditions were 3 min at 98°C; 20 cycles of 10 s at 98°C, 30 s at 62°C, and 45 s at 72°C; 10 min at 72°C. The PCR products were then purified using Agencourt AMPure XP Beads (Beckman Coulter, Beverly, MA, USA) with NEBNext Sizing Buffer (New England BioLabs) and eluted with 10 μL of 1× Tris-EDTA (TE) buffer. DNA concentration was quantified with the Qubit 2.0 Fluorometer using the Qubit dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA, USA), and the samples were then equimolarly mixed. For eliminating the risk of a higher proportion of short reads, the mixed sample was gel purified using the Zymoclean Gel DNA Recovery Kit, followed by additional purifications by AMPure Beads and QIAquick PCR Purification Kit, as described above. The number of DNA copies in the resulting sample was quantified using the Library Quantification Kit (Kapa Biosystems, Woburn, MA, USA), and the DNA library was subjected to emulsion PCR and subsequent sequencing on GS Junior platform according to the manufacturer's protocol (Roche, Basel, Switzerland).

Sequence processing and phylogenetic analyses—In total, the tag-encoded pyrosequencing yielded 61,592 raw sequences. All sequences with mismatches in tags were excluded, and the remaining reads were subjected to reduction of pyrosequencing noise (denoising) using Mothur 1.26.0 (Schloss et al., 2009). The obtained reads were then processed using the pipeline SEED ver. 2.0.54 (Větrovský and Baldrian, 2013). The sequences were demultiplexed based on their molecular identifier tags. A total of 23,870 sequences were retained in the data set after removal of sequences shorter than 380 bp. (These sequences and the associated metadata are available in the PlutoF repository at https://doi.org/10.15156/BIO/781237.) The resulting data set was trimmed to the sequence length of 380 bp, and the ITS1 region was extracted using ITSx ver. 1.0.11 (Bengtsson-Palme et al., 2013). The extracted ITS1 sequences were clustered to primary clusters using UPARSE implementation in USEARCH ver. 8.1.1861 (Edgar, 2013), with 97% similarity threshold (2621 detected chimeric sequences were excluded). All global singletons (471) were removed from the data set because most of these sequences are assumed to be artefactual (Tedersoo et al., 2010). For each primary cluster, a consensus sequence was constructed, using MAFFT ver. 7.222 (Katoh et al., 2009), based on the most abundant nucleotide at each position. The obtained consensus sequences were clustered a second time at 97%, as described by Kohout et al. (2015) and Krüger et al. (2015), using VSEARCH (Rognes et al., 2016). These secondary clusters are further referred to as "clusters". The taxonomic affiliation (i.e., affiliation to AMF) of consensus sequences representing the obtained 377 clusters was examined using BLAST against the GenBank and the UNITE, a manually curated database of annotated fungal ITS sequences (Kõljalg et al., 2013). The results of the BLAST searches are provided in Appendix S3.

To confirm taxonomic assignment and to estimate the diversity more accurately (i.e., closer to "reality", by merging the clusters belonging presumably to the same species), we aligned the sequences against a backbone database published by Krüger et al. (2012) using MAFFT version 7 (http://mafft.cbrc.jp/alignment/server/) and the slow, iterative refinement method (gap opening penalty 1.0, offset value 0.1). Five separate alignments (including also the reference sequences) were prepared for the following Glomeromycotina families/lineages: Gigasporaceae, Claroideoglomeraceae, *Rhizophagus*, *Funneliformis* + *Septoglomus* + *Glomus*, *Dominikia* + *Kamienskia* + remaining Glomeraceae (https://doi.org/10.15156/BIO/781238). Phylogenetic trees were obtained by distance analysis using the neighbour joining algorithm in MEGA7 (Tamura et al., 2011) with 1000 bootstrap replicates, the Kimura two-parameter model and the gamma shape parameter equalling 0.5. Operational taxonomic units (OTUs) of AMF were defined in a conservative manner as consistently separated monophyletic groups in the phylogenetic trees and confirmed by their characteristic sequence signatures in the alignments. AMF OTUs were designated after the major clade they belonged to, followed by a numerical index identifying each AMF OTU. To assess whether our sampling effort was sufficient to cover the AMF diversity at the field site, we constructed species accumulation curves showing the dependency of OTU number on the number of sampled plants and calculated species richness estimators (Chao 2 and ACE) using EstimateS 9.1.0 (Appendix S4).

Statistical analyses—Before statistical analyses, the resulting cluster and OTU tables were rarefied to the same sampling depth of 225 sequences (the lowest sequence number/sample) to enable sample comparison without bias due to different sampling depths and potential preferential amplification of certain tagged primers during multitag pyrosequencing. Statistical analyses were conducted for standardized numbers of both clusters and OTUs, with the same results; therefore, only the data for OTUs are further presented.

The general least-squares (GLS) models incorporated in the nlme package (Pinheiro et al., 2008) within the R environment

(R Core Team, 2014) were built to identify the main predictors of mycorrhizal root colonization and OTU richness (number of OTUs per sample), based on the following parameters: plant ploidy level (diploid vs. tetraploid) and soil characteristics (pH_{H2O}, CEC, C_{org}, P, N, K, Ca, Mg). Multicollinearities between predictor variables were checked by calculating the variance inflation factors (VIF); variables with VIF > 10 were excluded before model selection. The best GLS model was selected according to the corrected Akaike information criterion (AICc). Significance of the variables from the best fitting GLS model was also identified by averaging all models. Robustness of the best model was further evaluated by averaging models that fell into the 95% AICc confidence set. Beta coefficients (slopes) of individual models were weighted according to their Akaike weight across all models and evaluated as the mean ± 95% confidence intervals. Zero values were conservatively used for nonsignificant variables in individual models. Variables were considered significant when confidence intervals excluded zero values and were used in a best fitting model.

Before subsequent analyses of the structure of AMF OTU communities, the communities were standardized using the Hellinger transformation, which enables the use of linear-based ordinations for nonlinear data (Legendre and Gallagher, 2001). Bray–Curtis dissimilarity (Bray and Curtis, 1957) was used to examine AMF community structure. The effect of spatial structure on AMF diversity was taken into account by reducing the Euclidean distance matrix into spatial principle coordinates of neighbour matrices (PCNM) vectors that account for spatial autocorrelation at different scales (Borcard and Legendre, 2002). To address the relative impact of spatial distribution, plant ploidy, and soil characteristics on the AMF community structure, we used a multivariate ANOVA (PERMANOVA) as implemented in the Adonis routine of the vegan package of R (Oksanen et al., 2012). Adonis tests the significance of discrete and continuous factors based on permutations. Adjusted R^2 were calculated based on the Adonis results for each of the significant environmental variable. To illustrate the structure of AMF communities of individual plants, we performed hierarchical cluster analysis (hclust function in the stats package within the R environment) based on Bray–Curtis pairwise dissimilarity values (see above). We used the Ward clustering method, which minimizes within-cluster variance. The robustness of the obtained clusters was tested by a multiscale bootstrap approach (10,000 permutations) using updated pvclust function (pvclust package; Suzuki and Shimodaira, 2006; http://raw.github.com/nielshanson/mp_tutorial/master/taxonomic_analysis/code/pvclust_bcdist.R).

Plant growth in two inoculation treatments in a greenhouse experiment

Design and setup—The greenhouse pot experiment involved eight treatments based on a full-factorial combination of plant ploidy level (diploid vs. tetraploid), substrates (originating from microsites of diploids vs. tetraploids) and AMF inoculation (plants inoculated with native field substrates vs. plants inoculated only with microbial filtrates from these substrates). Each treatment combination was replicated eight times.

The plants were grown from seeds collected in 2008 from maternal plants of known ploidy level in the Sandberg mixed-ploidy population (Mráz et al., 2012b). For each ploidy level, eight seedlings originating from the same eight mother plants were used in each substrate × inoculation treatment. The seeds were surface-sterilized for 15 min in 10% v/v commercial bleach SAVO (Unilever ČR, Czech Republic; active substance NaClO) and germinated in Petri dishes. After 5 d, they were planted in multitrays with a sterile mixture of sand and garden substrate (3:1, v/v), grown for 10 days and then planted in experimental pots (diameter 16 cm) filled with the respective substrates (1550 mL).

Substrates were sampled in August 2011 from five microsites per cytotype (i.e., sites not larger than 1 m^2 that were preferentially occupied either by diploid or tetraploid plants) across the study site (Appendix S1) and were subsequently pooled according to the corresponding ploidy level. We pooled the substrates because we wanted to study the overall inter-cytotype differences in the response to native AMF. Owing to spatial heterogeneity in both substrate characteristics and AMF communities, we set-up homogeneous initial conditions to give the experimental plants a chance to interact with all AMF taxa present at the field site. In addition, we had to keep the number of soil samples reasonable due to the protection status of the study area. The collected sandy soil was sieved (<2 mm), homogenized and γ-sterilized (25 kGy; Bioster, Veverská Bítýška, Czech Republic). Three subsamples of each of the two cultivation substrates were analyzed for chemical characteristics. The results showed that the substrate from the microsites of the diploids was generally more fertile than the substrate from tetraploid microsites, as indicated by higher CEC, organic carbon content, and N, P, K, Ca, and Mg concentrations; no difference was recorded in pH value (Table 1B).

For each combination of plant ploidy level and substrate, two inoculation treatments were established. One half of plants was inoculated with 220 mL of the respective nonsterile field substrate (including root fragments), which was maintained at 4°C from sampling until inoculation (4 wk). We chose the inoculation with the nonsterile substrate in order not to ignore nonsporulating AMF and to involve lineages with different colonization strategies (predominant colonization from spores vs. infected roots). The inoculum was placed as a thin layer 5 cm below the surface of the substrate. The second half of the plants was provided with the same volume of the respective γ-sterilized substrate. As AMF inocula (native substrates in our case) always contain diverse communities of accompanying microorganisms, these plants were supplied with microbial filtrates of soil inocula (10 mL per plant) in the attempt to balance initial microbial communities between both inoculation treatments and introduce non-AMF microorganisms present in mycorrhizal inocula to the nonmycorrhizal treatment. The filtrate was prepared by filtration of a nonsterile soil suspension (1:10, w/v, 60 s homogenization by immersion blender, 1 h on a reciprocal shaker) through filter paper with a pore size of 15 μm to remove AMF propagules. We are aware that this approach does not allow separating organisms of similar or larger size than AMF spores such as representatives of micro- and mesofauna (Wagg et al., 2014); nevertheless, despite many efforts, there does not seem to be a way to establish a nonsterile cultivation experiment in which the treatments differ only by the presence of mycorrhizal fungi (Gryndler et al., 2018). Plants were cultivated in the greenhouse under natural daylight conditions, positioned randomly on the bench, and watered with distilled water to saturation as required. The temperature was maintained between 25°C (day) and 15°C (night) by an automatic ventilation/heating system.

Harvest—Plants were harvested before bolting after a 16-week cultivation (May–September 2011), before they became root-bound.

We counted the number of leaves and measured the length of the longest leaf. The shoots were then cut off, and the root systems were gently washed free of substrate. Small subsamples of the roots (~100 mg fresh mass) were taken from each plant and stained with 0.05% w/v trypan blue in lactoglycerol (Koske and Gemma, 1989) to assess mycorrhizal root colonization as described above. Shoot and root biomass was dried at 65°C, weighed, ground by an achate mill and analyzed for P concentration (Unicam UV4-100 spectrophotometer).

Data analysis—All data were analyzed with Statistica 12 software (StatSoft, Tulsa, OK, USA). Before the analyses, all data were checked for ANOVA assumptions (normality and variance homogeneity) and, if necessary, they were log (leaf number) or arcsine (root colonization)-transformed.

RESULTS

AMF in field-sampled roots of *C. stoebe* s.l.

Mycorrhizal root colonization—All sampled plants were colonized by AMF (Appendix S2) and average root colonization reached 88 ± 4% (mean ± SE). The cytotypes did not significantly differ in mycorrhizal root colonization ($F_{1, 28}$ = 0.07, P = 0.79). DSE colonization reached 11 ± 2% (Appendix S2), again without significant intercytotype difference ($F_{1, 28}$ = 0.17, P = 0.68). Rhizosphere soil of diploids showed significantly higher organic C content and N and Mg concentrations (Table 1A; for F and P values, see Appendix S5A); none of the measured soil chemical variables, however, affected the level of AMF colonization according to GLS analysis.

Richness and composition of AMF communities—After the second clustering at the 97% sequence similarity threshold, we retrieved 377 clusters involving more than one sequence with affinity to the Glomeromycotina (Appendix S6), which were assigned to 49 phylogenetically supported AMF OTUs (Appendix S7). AMF richness varied from 4 to 18 AMF OTUs per individual host plant, with an average of 11 taxa (Appendix S8). Most OTUs were affiliated with Glomeraceae, only two with Gigasporaceae and one with Claroideoglomeraceae (Appendix S9). The most abundant OTU, identified as *Rhizophagus irregularis* (OTU 45), was represented by 28% of all reads, followed by *Dominikia* sp. (OTU 6) with 12% of sequences, and two Glomeraceae spp. (OTU 19 and OTU 22) with 12 and 8% of sequences, respectively. Thirty-three OTUs were each represented by less than 1% of all sequences (for the complete OTU summary, see Appendix S9). Species accumulation curves (Appendix S4) showed that our sampling effort was sufficient to characterize most of the AMF taxa inhabiting *C. stoebe* roots at the Sandberg site. According to the richness estimators Chao 2 and ACE, only the presence of one or two additional AMF species might be predicted for the site.

As revealed by GLS, neither ploidy nor environmental variables affected AMF richness. The PERMANOVA analysis showed that the composition of AMF communities was significantly affected only by the spatial distribution within the field site (P < 0.01) and pH level (P < 0.05), explaining 12.4% (AdjR^2) and 2.3% of the total variability, respectively. Hierarchical clustering of AMF communities of individual host plants revealed four moderately supported clusters (the approximative unbiased P values [AU] above 50%) and eight highly supported subclusters with highly similar AMF communities (minimal AU P > 88%, Fig. 1A). Similar AMF communities (Fig. 1A) were partially

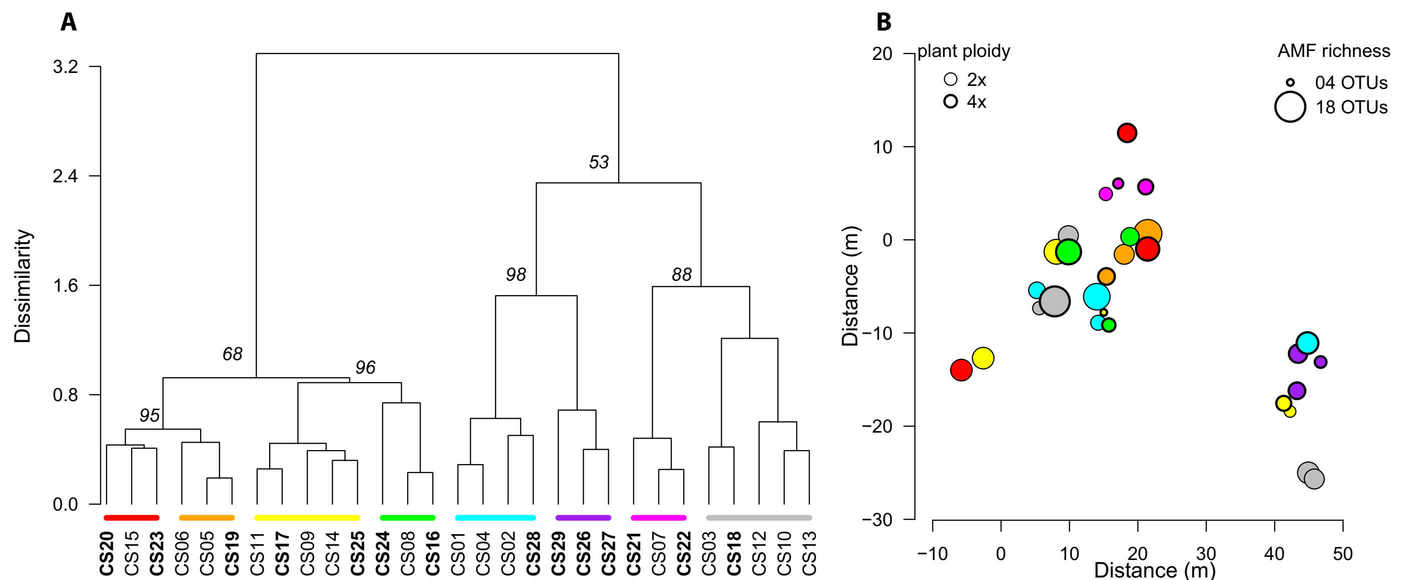

FIGURE 1. Structure, diversity, and spatial distribution of arbuscular mycorrhizal fungi (AMF) communities in roots of 29 diploid (2*x*) and tetraploid (4*x*) plants of *Centaurea stoebe* s.l. at Sandberg hill. (A) Structure of AMF communities detected by cluster analysis (Ward method) based on Bray–Curtis dissimilarity values. Codes of diploid host plants are denoted by regular letters, while tetraploid host plants are denoted by bold letters. Different colors highlight eight highly supported clusters of AMF communities revealed after bootstrapping. The numbers in italic above the nodes represent the approximate unbiased P values in % after 10,000 permutations. (B) Spatial distribution and diversity of AMF communities. Eight highly supported clusters are denoted by different colors as in A, while the taxonomic richness of AMF communities, i.e., absolute number of detected OTUs, is delineated by the different size of the circles.

TABLE 2. Percentage of colonization of *Centaurea stoebe* roots by arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) in the greenhouse experiment. None of the variables was significantly influenced by ploidy level, substrate, or their interaction. The data represent means (± SE) of eight plants inoculated with native field substrates (mycorrhizal treatment). In roots of the plants inoculated only with microbial filtrates from the native field substrates (nonmycorrhizal treatment), no AMF and DSE structures were observed.

Ploidy level	Substrate	AMF colonization			DSE colonization (%)
		Total (%)	Arbuscular (%)	Vesicular (%)	
2*x*	2*x*	98.1 ± 0.5	83.5 ± 3.9	73.5 ± 3.7	7.0 ± 2.5
	4*x*	94.9 ± 1.2	72.9 ± 3.3	69.3 ± 4.8	4.3 ± 2.3
4*x*	2*x*	98.0 ± 0.9	79.8 ± 3.5	67.4 ± 5.8	7.5 ± 2.2
	4*x*	97.5 ± 1.0	77.8 ± 3.5	68.8 ± 3.9	5.1 ± 1.7

TABLE 3. Effects of ploidy level (diploid [2*x*] vs. tetraploid [4*x*]), substrate (from microsites inhabited by diploids vs. tetraploids), inoculation (plants inoculated with native field substrates [mycorrhizal treatment] vs. plants inoculated only with microbial filtrates from these substrates [nonmycorrhizal treatment]) and their interactions on *Centaurea stoebe* s.l. growth and P uptake in the greenhouse experiment according to a three-way ANOVA. Significant effects of the independent variable are given in bold, with *F* values and coefficients of determination (R^2; in parentheses).

Factor	df	Leaf number	Longest leaf length	Shoot dry mass	Root dry mass	Root : shoot ratio	Shoot P concentration
Ploidy level	1	ns	**18.08** ** **(0.12)**	**6.57** * **(0.03)**	**4.38** * **(0.02)**	**12.35** ** **(0.13)**	ns
Substrate	1	**61.58** ** **(0.50)**	**70.70** ** **(0.48)**	**126.31** ** **(0.59)**	**90.15** ** **(0.40)**	ns	ns
Inoculation	1	**4.52** * **(0.04)**	ns	**16.61** ** **(0.08)**	**56.50** ** **(0.25)**	**22.47** ** **(0.23)**	**130.76** ** **(0.67)**
Ploidy × substrate	1	ns	ns	**5.32** * **(0.02)**	ns	ns	ns
Ploidy × inoculation	1	ns	ns	ns	**7.19** * **(0.03)**	ns	ns
Substrate × inoculation	1	ns	ns	ns	**5.87** * **(0.03)**	ns	ns
Ploidy × substrate × inoculation	1	ns	ns	ns	ns	ns	ns

Notes: Significant effect at *$P < 0.05$; **$P < 0.001$; ns, nonsignificant effect.

spatially aggregated but without any pattern with regard to the ploidy of host plant (Fig. 1B).

Plant growth in the inoculation treatments in the greenhouse

Mycorrhizal root colonization—No AMF structures were observed in roots of plants inoculated with microbial filtrates only, while all plants inoculated with nonsterile native substrates were heavily colonized by AMF with high frequency of arbuscules and vesicles (Table 2). Mycorrhizal root colonization ranged from 92 to 100% (mean ± SE, 97 ± 1%), and the level of colonization did not significantly differ between ploidy levels and substrates (Table 2; for *F* and *P* values, see Appendix S5B). Taking into account this clear-cut pattern in AMF root colonization in both inoculation treatments, we hereafter refer to the treatment inoculated with microbial filtrates as "nonmycorrhizal" and the treatment inoculated with nonsterile native substrates as "mycorrhizal". The plants in the mycorrhizal treatment also had a low level of DSE colonization (on average 6 ± 1%), also without any significant differences between ploidy levels and substrates (Table 2; Appendix S5B).

Growth and P nutrition—Plant growth was more affected by the substrate than by ploidy level and AMF inoculation (Table 3; for *F* and *P* values, see Appendix S5C). Shoot dry mass was correlated with number of leaves and with length of the longest leaf ($r = 0.70$ and $r = 0.71$, respectively, $P < 0.001$); therefore, only the data on shoot dry mass are presented. While ploidy level and inoculation accounted for only 3% and 8% of the total variation in shoot dry mass, respectively, the value rose to 59% for the effect of the substrate. The plants produced generally more biomass in the more fertile substrate originating from microsites of diploids (Table 3, Fig. 2). Despite differences in the fertility of both cultivation substrates (Table 1B), P concentration in plant shoots was not affected by the substrate origin (Table 3, Fig. 3).

Considering the effect of ploidy level, tetraploids of *C. stoebe* produced longer leaves (Table 3), which contributed to their significantly higher shoot dry mass compared to their diploid counterparts (Fig. 2). The only growth characteristics not influenced by ploidy level was number of leaves (Table 3). Shoot dry mass was also significantly influenced by a ploidy × substrate interaction. While in the less fertile substrate the cytotypes did not significantly differ, in the more fertile substrate tetraploids produced more shoot biomass than diploids. As documented by a higher root to shoot ratio, diploids invested significantly more into the root biomass, regardless of the substrate (Table 3, Fig. 2). The cytotypes did not differ in phosphorus concentration in their shoots (Table 3).

The mycorrhizal plants inoculated with nonsterile native field substrates from either diploid or tetraploid microsites had significantly lower aboveground biomass (on average by 25%), without any inter-cytotype difference (Table 3, Fig. 2). In spite of this growth reduction, mycorrhizal plants had significantly higher shoot P concentrations, irrespective of ploidy level and substrate treatment (Table 3, Fig. 3). As a result, mycorrhizal plants also had significantly

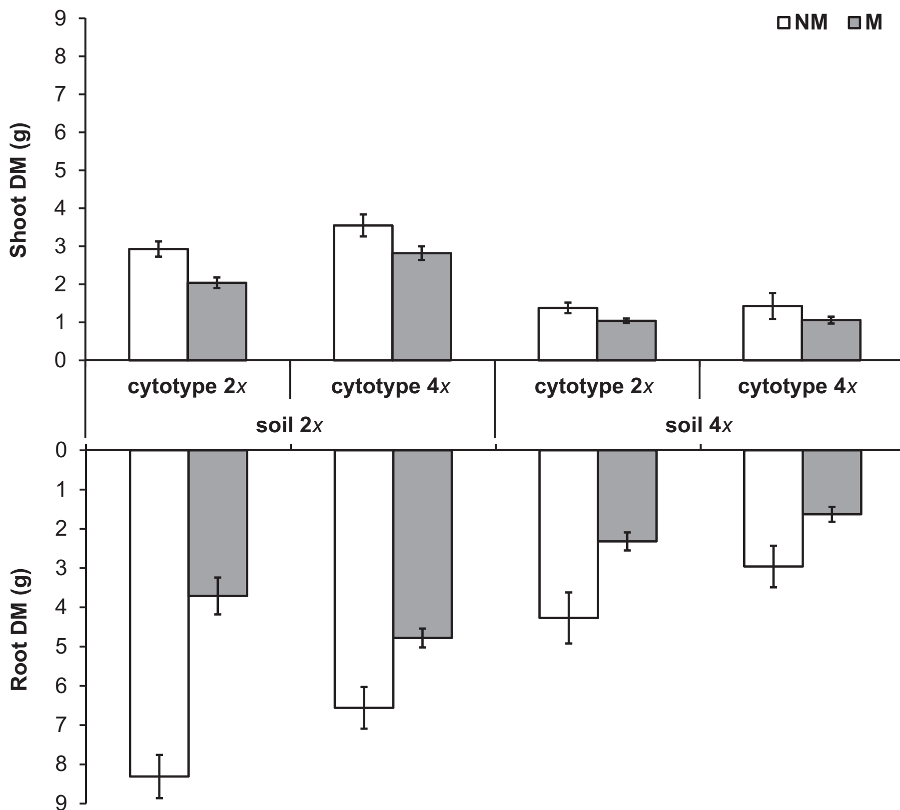

FIGURE 2. Shoot and root dry mass (DM) of *Centaurea stoebe* plants from the greenhouse experiment as affected by ploidy level (diploid [2*x*] vs. tetraploid [4*x*]), substrate (from microsites inhabited by diploids vs. tetraploids) and inoculation (plants inoculated with native field substrates [M] treatment vs. plants inoculated only with microbial filtrates from these substrates [NM] treatment). The columns represent means (± SE) of eight replicates.

higher P content (total P uptake per plant) than their noninoculated counterparts (data not shown); on average, P concentrations and contents in the shoots of noninoculated plants were more than 40% and 20% lower, respectively. The inoculation treatment also affected biomass allocation, with the mycorrhizal plants investing less into the roots (Table 3, Fig. 2). Root biomass was significantly influenced by the interaction of substrate and inoculation treatment (Table 3, Fig. 2) as we observed a greater difference in root biomass between the mycorrhizal and nonmycorrhizal inoculation treatments when grown in diploid-microsite soil than when grown in tetraploid-microsite soil. Root biomass was also significantly affected by an interaction between inoculation and ploidy (Table 3; Fig. 2). In the nonmycorrhizal treatment, diploid plants had greater root dry mass than did tetraploid plants, whereas diploid and tetraploid root dry mass did not differ significantly after the mycorrhizal inoculation.

DISCUSSION

AMF communities do not reflect cytotype structure of *C. stoebe* population

Higher soil fertility recorded at microsites occupied by diploid plants is consistent with microhabitat preferences reported for *C. stoebe* cytotypes at the same mixed-ploidy population by Mráz et al. (2012b). In spite of this spatial fine-scale habitat segregation, we found no evidence that coexisting cytotypes of *C. stoebe* differ either in the level of mycorrhizal root colonization or in AMF communities. Absence of an inter-cytotype difference in mycorrhizal colonization (taken as a proxy for intraradical development of AM symbiosis) was also found in three other plant species previously studied (Sudová et al., 2010, 2014). Thus ploidy-associated changes in cell size and cell wall structure (Do Valle et al., 1988; Beaulieu et al., 2008) do not seem to decrease or inhibit penetration of fungal hyphae into root tissues.

The present study is the first to assess potential differences in assemblages of AMF between co-existing cytotypes of the same species complex. To the best of our knowledge, this issue has only been studied for orchid mycorrhizal fungi where a distinct segregation of mycorrhizal symbionts was found for co-existing cytotypes of *Gymnadenia conopsea* s.l. (Těšitelová et al., 2013), indicating that this contributed to niche partitioning between the cytotypes. In the case of *C. stoebe* s.l., diploid and tetraploid individuals differed in their microhabitat preferences, but this differentiation was not coupled with changes in richness or composition of AMF communities in their roots. Therefore, it is unlikely that ploidy-specific communities of AMF contributed to the co-existence of the cytotypes in the contact zone.

The spatial distribution of the sampled plants at the study site significantly explained about 12% of the total variability in AMF community composition. In contrast, ploidy did not affect the structure of AMF communities and soil chemistry had only a marginal effect on it (pH explaining 2.3% of total variability). In spite of microhabitat differentiation, the differences between microsites inhabited by diploids and tetraploids were likely too small to induce a significant shift in the structure of AMF communities (e.g., pH range was relatively narrow, from 7.3 to 7.6). The spatial effect on AMF community composition might have been caused by an unmeasured environmental variable such as soil structure or soil moisture. On the other hand, it is becoming obvious that AMF community composition is not exclusively driven by deterministic process and that stochastic processes might be even more important (Caruso et al., 2012; Powell and Bennett, 2016). Spatially structured and patchily distributed AMF communities have also been recorded in other local-scale studies in relatively homogeneous environments (Rosendahl and Stukenbrock, 2004; Mummey and Rillig, 2008). We assume that in the absence of strong environmental gradients the relative importance of stochasticity in driving AMF communities is higher on a small scale (up to several tens of meters, as in our case). At the similar fine-spatial scale, a random distribution and the lack of dispersal limitation have also been evidenced for soil eukaryotic microbes in general (Bahram et al., 2016). Interestingly, a higher relative importance of stochasticity has been reported for fungal communities associated with roots than with soil (Beck et al., 2015).

Notably, AMF communities in *C. stoebe* roots were dominated by representatives of the Glomeraceae, while other phylogenetic lineages were scarce. Members of the Glomeraceae show a number of features matching with a ruderal life-history strategy (Chagnon et al., 2013), including fast growth, early and abundant sporulation, high investment into intraradical relative to extraradical hyphae (Hart and Reader, 2002; Powell et al., 2009), frequent hyphal fusion enabling mycelium re-establishment after disturbance (De la Providencia et al., 2005), or ability to colonize plants via fragments of hyphae or colonized roots (Hart and Reader, 2002). Accordingly, strong predominance of the Glomeraceae within the AMF community has been reported from habitats subjected to periodic disturbance, such as agricultural soils, sand dunes, or regularly flooded sites (e.g., Daniell et al., 2001; Wang et al., 2011; Borriello et al., 2012; Estrada et al., 2013). We therefore ascribe the predominance of Glomeraceae at our sampling site to the instability of sand substrates, in combination with previous and recent human-induced mechanical disturbances (sand exploitation, hiking, and fossil hunting).

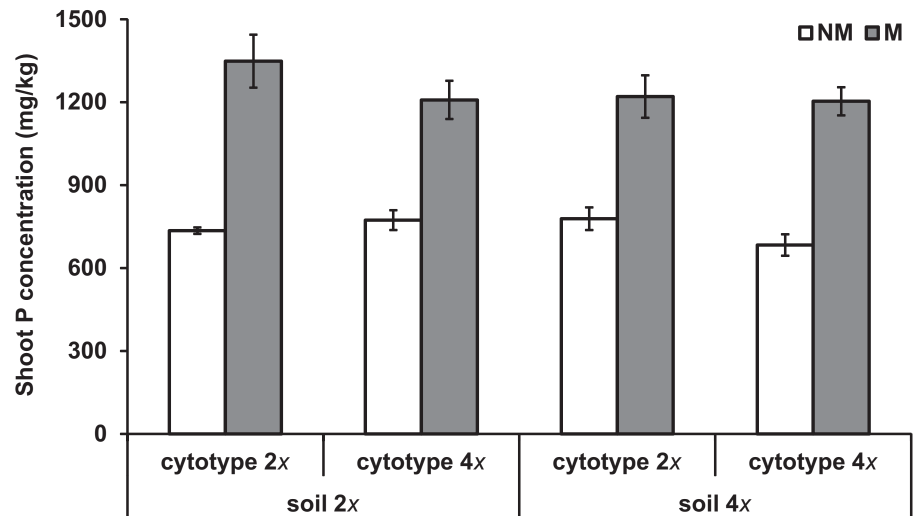

FIGURE 3. P concentration in *Centaurea stoebe* shoots from the greenhouse experiment as affected by ploidy level (diploid [2*x*] vs. tetraploid [4*x*]), substrate (from microsites inhabited by diploids vs. tetraploids) and inoculation (plants inoculated with native field substrates [M] treatment vs. plants inoculated only with microbial filtrates from these substrates [NM] treatment). The columns represent means (± SE) of eight replicates.

Negative growth response of *C. stoebe* to inoculation with native field substrates

Considering the sandy nature of the study site and low available phosphorus concentrations in the substrate, we expected improved plant growth and P uptake in response to AMF inoculation in our greenhouse experiment. In contrast, growth of the plants inoculated with native AMF was significantly depressed, irrespective of ploidy level and substrate. The lack of differences in the effects of AMF originating from diploid vs. tetraploid microsites of *C. stoebe* can likely be attributed to the overall similarity of AMF communities in roots of the two cytotypes as revealed by pyrosequencing. Plant growth depressions due to AMF colonization are not rare (Klironomos, 2003) and are traditionally explained as a consequence of imbalance between carbon costs (drain to the mycobiont) and benefits gained in the form of improved nutrient acquisition and/or increased resistance to biotic or abiotic stresses (Johnson et al., 1997). Negative mycorrhizal growth responses are often reported under nitrogen-limiting conditions due to N retention in fungal tissues (Correa et al., 2014; Johnson, 2010; Johnson et al., 2015); however, *C. stoebe* plants at Sandberg are P-limited (mean N:P ratio ± SE: 17.9 ± 0.7, n = 6). In spite of mycorrhizal growth depression, phosphorus uptake by *C. stoebe* plants was improved by AMF inoculation, regardless of ploidy level and substrate treatment. The fact that AMF hyphae partially took over the role of roots in nutrient acquisition is also obvious from the significantly lower investment of inoculated plants into root biomass (savings in carbon costs of root production are a common response to root colonization by AMF; Smith et al., 2011). We suppose that the reason that increased plant P uptake was not mirrored in growth enhancement can be due to the very high level of mycorrhizal root colonization. Consistent with data from the field, the roots of experimental plants were highly colonized by AMF (92–100%), regardless of ploidy level and substrate. Considering this significant sink for plant carbon, we hypothesize that an AM-mediated increase in P uptake did not outweigh considerable costs for maintaining AM symbiosis. It is, however, also possible that the primary benefit derived from AMF colonization by *C. stoebe* in the field is not nutritional but rather protective. Ruderal AMF communities, formed predominantly by representatives of Glomeraceae, were shown to provide more efficient protection against phytopathogens than other AMF lineages (Maherali and Klironomos, 2007).

When interpreting the observed growth depression in the mycorrhizal treatment, we also need to remember that it is virtually impossible to produce nonmycorrhizal controls with identical composition of microbial communities as in the mycorrhizal treatment (Gryndler et al., 2018). We therefore cannot completely rule out two alternative explanations considering possible differences in the composition of non-AMF microbial communities in both inoculation treatments. First, the growth depression effect of our mycorrhizal treatment could have been caused by an unintentional introduction of soil pathogens with the AMF inoculum. However, the roots of *C. stoebe* did not show any symptoms such as necrotic lesions, and trypan blue staining revealed only the presence of AMF and DSE in roots of the mycorrhizal plants. Colonization by DSE fungi (likely introduced as resting microsclerotia in root fragments) was very low and unlikely to be related to the observed growth depression. Second, the observed differences in the growth between the plants from both inoculation treatments could also be caused by putative growth-enhancing effects of microbial communities established from the microbial filtrates in the nonmycorrhizal treatment.

Centaurea stoebe cytotypes do not differ in their response to inoculation with native AMF

Lack of a differential effect of AMF on *C. stoebe* cytotypes contradicts the hypothesis of a generally increased importance of AM

symbiosis in polyploids due to their higher nutritional requirements (Segraves and Anneberg, 2016). By theory, this differentiation in nutritional demands should more likely occur between diploids and higher polyploids and should correspond with C-value data (holoploid genome size), which reflect the number of phosphorus and nitrogen atoms needed to build the DNA of every cell (Sterner and Elser, 2002). Nevertheless, a comparison of AMF effects on polyploid complexes studied in the recent and previous experiments (Sudová et al., 2010, 2014) does not support this hypothesis. Although a ploidy-specific mycorrhizal growth response was observed only for the species with the highest C-value (*Aster amellus*, 2C-value ~7.9 pg vs. *C. stoebe,* ~1.6 pg/2C, *Campanula gentilis,* ~2.3 pg/2C, *Pimpinella saxifraga,* ~3.8 pg/2C; our data and Bennett and Leitch, 2012), the diploids of *A. amellus* profited more than the hexaploids from being mycorrhizal.

Might native AMF contribute to the non-invasiveness of *C. stoebe* in its primary range?

The very high mycorrhizal root colonization observed in *C. stoebe* roots in our study (88% in field samples and 97% in greenhouse experiment) contrasts with considerably lower values reported for *C. stoebe* tetraploids in most studies from the introduced range (mean range 15–64%, with prevailing lower values; Marler et al., 1999b; Zabinski et al., 2002; Walling and Zabinski, 2004, 2006; Callaway et al., 2004a; Carey et al., 2004; Klein et al., 2006; Meiman et al., 2006; Emery and Rudgers, 2012), except for the studies of Harner et al. (2010) and Lekberg et al. (2013). Interestingly, except for the study by Walling and Zabinski (2006), AMF have been found to either increase the growth or the competitiveness of *C. stoebe* tetraploids from the introduced range (Marler et al., 1999a; Zabinski et al., 2002; Callaway et al., 2004a; Carey et al., 2004; Harner et al., 2010; Emery and Rudgers, 2012). When we consider the mentioned differences in mycorrhizal root colonization and mycorrhizal growth response, our results raise the question whether AMF may contribute to the non-invasiveness of *C. stoebe* in native communities in the primary range. However, without a direct comparison of the effects of AMF on *C. stoebe* populations from both native and introduced ranges, this explanation remains purely speculative. A link between plant–soil feedback and the success of *C. stoebe* tetraploids in North America and its control in native European range was, however, outlined already by Callaway et al. (2004b), who demonstrated stronger inhibitory effects of soil biota in general on *C. stoebe* in native European soils compared to soils from the invasive range.

Inter-cytotype differences in *C. stoebe* performance

Collins et al. (2011), Henery et al. (2010), and Mráz et al. (2014) found no significant difference in aboveground biomass production between diploid and tetraploid cytotypes of *C. stoebe*. Our results nevertheless demonstrate that whether ploidy divergence in plant growth occurs or not is significantly influenced by substrate fertility. While no inter-cytotype difference in shoot biomass of *C. stoebe* was recorded in the less fertile substrate, better growth of the tetraploids was manifested in the more fertile substrate. The enhancement of inter-cytotype differences in plant growth with increasing soil fertility was also demonstrated for diploid-hexaploid *Aster amellus* (Sudová et al., 2014), which confirms higher plasticity of the polyploids in resource capture. Furthermore, we observed significantly higher root biomass and higher root to shoot ratios for the diploids, while Collins et al. (2011, 2013) reported the opposite and argued that larger root systems may increase competitive ability of tetraploids. A direct comparison of different studies is, however, difficult due to considerable inter-population variability (Collins et al., 2011; Hahn and Müller-Schärer, 2013; Mráz et al., 2014) and varying cultivation conditions (duration of the experiment, cultivation period, fertility of the substrate, harvest timing at different ontogenetic stage), which have strong effects on biomass production and allocation.

CONCLUSIONS

In addition to altering plant anatomy, morphology and physiology, polyploidization in angiosperms may also alter biotic interactions, including those with soil microorganisms. In our study, we tested whether two cytotypes of *Centaurea stoebe* differ in the composition of AMF communities and whether the cytotypes respond differentially to root colonization with native AMF in a greenhouse experiment. Contrary to our expectations, we found no evidence for ploidy-specific segregation in the interaction of *C. stoebe* with AMF in the field or in the greenhouse. In spite of a significant increase in P uptake, AMF inoculation resulted in a considerable reduction of plant growth, regardless of ploidy level. We thus hypothesize that the negative growth response to AMF inoculation could be a mechanism contributing to the control of the population growth and density of *C. stoebe* in its native range. Verification of this hypothesis, however, requires further testing, both under experimental and in situ conditions. In light of the neutral or positive effects of AMF on growth or competitiveness reported for the tetraploid cytotype from the introduced range, comparing the structure and composition of AMF communities in native and in introduced tetraploid populations and assessing their growth responses to native and foreign AMF assemblages are of high interest.

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DATA ACCESSIBILITY

454-sequences and metadata: PlutoF repository https://doi.org/10.15156/BIO/781237.

ITS1 alignments: PlutoF repository https://doi.org/10.15156/BIO/781238.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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