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ORIGINAL ARTICLE

Elevational distribution and occurrence of arbuscular mycorrhizal fungi in non-host *Carex capillacea*

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Abstract

Little is known about Arbuscular mycorrhizal (AM) fungal colonization and community composition in non-mycorrhizal (NM) plants, especially along elevational gradients. This study explores this question using a NM plant, *Carex capillacea*, at Mount Segrila, Tibet. Here, *C. capillacea*, its rhizosphere soil, and the neighboring mycotrophic plant *Poa annua* were sampled at four elevations to evaluate and compare their AM fungi colonization and communities. The results showed that AM fungal colonization density of *C. capillacea* was negatively correlated with elevation and biomass of total NM plants per quadrat. AM fungal diversity and community composition between *C. capillacea* and *P. annua* showed a similar pattern. In addition, elevation and soil did not significantly influence the AM community in *C. capillacea*, while they were important abiotic factors for assemblages in rhizosphere soil and *P. annua*. These findings support that a broad array of AM fungi colonize the root of *C. capillacea*, and a mycelial network from a co-occurring host plant might shape the AM fungal communities in *C. capillacea* along the elevation gradient. The co-occurrence patterns of AM fungi associated with non-mycotrophic species and adjacent mycotrophic species have important implications for understanding AM fungal distribution patterns and plant–AM interactions.

Keywords Arbuscular mycorrhizal fungi · Non-mycorrhizal plant · Root colonization · Elevation gradient · Diversity · Mycelial network

Introduction

Arbuscular mycorrhizal (AM) fungi are important soil microorganisms that form mutualistic associations with more than 70% of terrestrial vascular plants (Brundrett 2009; Brundrett and Tedersoo 2018). These obligate biotrophs generally improve nutrient acquisition and alleviate biotic and abiotic stresses to the host plants (Smith and Read 2008; Kiers et al. 2011), and thus play a crucial role in maintaining the productivity and diversity of plant communities (van der Heijden et al. 1998; Li et al. 2015; Powell and Rillig 2018). Still, a limited number of vascular plants, especially members of Cyperaceae, Amaranthaceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, and Juncaceae, are considered as "non-mycorrhizal" or "non-mycotrophic" (NM) families (Tester et al. 1987; Brundrett 2009). These NM plants typically employ alternative nutritional strategies such as structural adaptations (e.g., dauciform and proteoid roots), parasitism, and carnivory in response to nutrient limitation (Lamont 1982, 2003; Skene 1998). From an evolutionary perspective, their progenitors had already experienced the loss or suppression of functional mycorrhizal symbiosis as some of them were confined to phylogenetically independent plant families (Vigneron et al. 2018). These species are generally thought to have a low affinity with mycorrhizas under natural conditions (Miller 2005).

The diagnosis of mycotrophic plants is often defined by microscopic features of roots (e.g., arbuscules), however,

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which may cause ambiguous distinction of mycorrhizas for plant families, genera, and even species (Brundrett 2009). An increasing number of studies has shown that the NM plants often could be weakly colonized by AM fungi forming rudimentary AM phenotype structures such as hyphae, vesicles, and even rare arbuscules when they co-occur with mycorrhizal plants (Veiga et al. 2013; Cosme et al. 2018; Wang et al. 2021). Still, only a few reports indicated this establishment of AM symbiosis had negative or positive effects on the growth of NM plants (Williams et al. 1974; Allen et al. 1989; Lekberg et al. 2015), suggesting that we know surprisingly little about AM fungi in a putative NM species.

Cyperaceae are typified by the genus *Carex* (over 200 species) and have a worldwide distribution (Miller 2005), and Cyperaceae are used to be classified as a NM family within the mycotrophically diverse order, the Poales. Muthukumar et al. (2004) investigated data from 221 sedge members, however, and found mycotrophic and NM species accounted for 40% and 49%, respectively. Nonetheless, intensive studies of mycorrhizal colonization in the genus *Carex* have shown that the main colonization structures are hyphae, vesicles, and few arbuscular structures (Miller et al. 1999; Muthukumar and Udaiyan 2002; Johnson et al. 2004), indicating likely passive mycorrhizal colonization in roots of certain species. Additionally, the research comparing the growth of some members of Cyperaceae alone or together with mycorrhizal host plants under greenhouse conditions has shown that the cyperaceous species could not independently support an active mycorrhizal mycelial network or propagule production (Ruotsalainen and Aikio 2004; Zhang et al. 2019). Therefore, the colonization experienced in the roots of some species of Cyperaceae is more likely a result of part of the surroundings mycelial network, and colonization intensity may be based on the activity of the mycotrophic plant community (Fitter et al. 1998; Zhang et al. 2019). Miller et al. (1983) found the level of colonization of *Atriplex confertifolia* (Amaranthaceae) was related to the degree of adjacent mycorrhizal host plant cover. Nevertheless, in some wet or disturbed habitats, the Cyperaceae adjoining a group of mycotrophic plants had rare mycorrhizal colonization, suggesting that climatic and edaphic conditions also might be constraining (Muthukumar et al. 2004; Choudhury et al. 2010). Thus, the question remains, “what is the most important driver for AM colonization in Cyperaceae?”.

Cyperaceae often are found to have a wide distribution in alpine ecosystems where AM fungi also exist, climate change is dramatic, and biotic turnover over short spatial scales is pronounced (Tester et al. 1987; Li et al. 2016). To date, a variety of studies have investigated the response of AM fungi in host plants to elevational gradients, suggesting that elevational gradients significantly influence mycorrhizal colonization (Gai et al. 2012; Lugo et al. 2012), spore density, AM fungi diversity (Gai et al. 2012), and community composition (Guo et al. 2020; Li et al. 2020). Additionally, our previous pot experiment showed that when *C. capillacea* was grown alone or adjoining the mycorrhizal host species *Medicago sativa* at two elevational sites, neighboring host plants guaranteed the presence of an active AM fungal network but may had more limited effects on the AM colonization of *C. capillacea* than alpine environmental conditions (Zhang et al. 2019). Hence, relative to neighboring host plant presence, alpine conditions might be a key driver for AM colonization and even community diversity in the roots of Cyperaceae along environmental gradients.

Mount Segrila is situated in the Tibetan Plateau at the convergence of the east Nyainqentanglha and east Himalaya ranges. It comprises a typical alpine ecosystem with various climate types and great differences in vertical zonal distribution. Cyperaceae and mycotrophic plants (e.g., the genus *Poa*) have a wide distribution there; thus, it is an ideal site for investigating the ecological responses of AM fungi in non-host roots to environmental changes.

In the present study, AM fungi in the roots of the putative NM Cyperaceae species *C. capillacea*, its rhizosphere soil, and the neighboring host plant *Poa annua* were investigated at Mount Segrila, to evaluate the effects of alpine environmental conditions along an elevational gradient, and mycotrophic plant presence on AM fungal colonization and community composition of *C. capillacea* roots. We hypothesized that (i) AM fungal colonization and diversity in roots of *C. capillacea* would be lower than that in the co-occurring *P. annua*, and (ii) AM fungal colonization and community composition in the roots of *C. capillacea* mainly would be affected by alpine environmental conditions, followed to less extent by neighboring host plants.

Materials and methods

Site description, soil, and plant sampling

The research was carried out along an elevational transect on the east slope of Mount Segrila (29°21′–29°50′N, 94°28′–94°51′E), where the natural tree line is 4000–4200 m a.s.l. and the peak is 5300 m a.s.l. There are four distinct vegetation types along the elevational gradient: temperate coniferous and broad-leaved mixed forest, montane dark coniferous forest, sub-alpine shrub meadow, and alpine meadow (Li et al. 2020). As a result, we selected four elevations (3178, 3904, 4285, and 4510 m, respectively) to sample. Details of the sampling locations are provided in Fig. S1 and Table S1.

Plants and soil were sampled in July 2017 during the active growth period. Three sample types, *C. capillacea*, its rhizosphere soil, and *P. annua* were collected from twelve sampling quadrats (each 1 × 1 m) in total at four elevations. Five soil monoliths (10 × 10 cm, 0–15 cm depth) were collected from each quadrat. Three sample types were collected from monoliths of each quadrat and mixed respectively as samples of each quadrat ($n = 36$, 4 altitude × 3 quadrats/replicates × 3 sample types). The minimum distance between any two quadrats was 5 m. The aboveground parts of other plants also were harvested in each quadrat and the numbers of different functional types were recorded (i.e., groups of mycotrophic and putative non-mycotrophic species based on previously published data). All plant samples were carefully washed with distilled water. Shoots were dried at 65 °C for 48 h and weighed for biomass. The roots of *C. capillacea* and *P. annua* were stored at − 20 °C for AM colonization and molecular analyses. The soil samples were screened (2.0 mm). The rhizosphere soil of *C. capillacea* was stored at − 20 °C for AM fungi community analysis, and the monolith soil was air dried and stored at room temperature for the determination of chemical properties.

Soil chemical properties and assessment of AM colonization

The soil pH was measured at a 1:2.5 ratio of soil–water suspension with a glass electrode. Soil organic carbon (SOC) was determined with the $K_2Cr_2O_7$-H_2SO_4 method (Walkley and Black 1934). Total carbon (TC) and total nitrogen (TN) were determined using an elemental analyzer (EA1108, Fisons Instruments SpA, Milan, Italy). Available phosphorus (AP) was extracted with $NaHCO_3$ and analyzed using a Cary 50 UV–vis spectrophotometer (Agilent Technologies, Santa Clara, CA) (Olsen 1954).

Root segments for estimation of AM colonization were cleared in 10% KOH and stained in 0.05% (v/v) Trypan blue in lactoglycerol (modified from Phillips and Hayman 1970). Root colonization rates of hyphae, vesicles, arbuscules, and total abundance of mycorrhizal structures were carefully estimated using the magnified-intersections method (McGonigle et al. 1990) with at least 300 intersections from 30 root segments of ca. 1 cm length at × 200 magnification per root sample.

Molecular analysis

After randomly selected roots were ground fresh in liquid N, root (ca. 0.1 g) and rhizosphere soil (ca. 0.5 g) samples were used to extract genomic DNA using the Plant/Soil Rapid Genomic DNA Kit (Tiangen biotech, Beijing, China) following the manufacturer's protocols. The extracted DNA was stored at − 20 °C and normalized to equivalent concentrations according to quantification by NanoDrop ND-1000 UV–Vis spectrophotometer for the following nested PCR procedure. The PCR procedure was the same as in Xiang et al. (2016). The first PCR was performed using primer set AML1/AML2 (Lee et al. 2008). The PCR product was diluted and then amplified as the template in a second PCR using the primer set AMV4.5NF/AMDGR (de Souza et al. 2004). PCR products were used to check for results on a 1.5% agarose gel and then purified with a PCR Purification Mini Kit (Aidlab Biotechnologies, Beijing, China). Each sample contained a variable length (8–12 bp) unique error-correcting barcode to carry out sequencing on an Illumina MiSeq System (Illumina Inc., San Diego, CA) running in paired-end 2 × 300 bp mode.

Sequences including nucleotides mismatched within the barcode and primer regions and sequences shorter than 50 bp and with an average quality score lower than 20 were discarded. Assembling of available paired-end reads was by two criteria: (1) The minimum overlapping length between the forward and reverse reads was 10 bp, and (2) the maximum mismatch ratio of the overlapping region was 0.2. Then, after filtering and denoising, the sequences were clustered to the same OTU with 97% similarity, and the most abundant sequence of each OTU was used as the representative sequence. Subsequently, non-Glomeromycota sequences were detected by manual blasting against the GenBank nonredundant nucleotide database. Representative sequences were clustered by phylogenetic analysis incorporating reference sequences from both the GenBank and *MaarjAM* databases (version 0.8.1, http://www.maarjam.botany.ut.ee/). According to the results of the species annotation, the sequences also were used to construct a maximum likelihood tree using non-parametric bootstrapping in MEGA 6 (Kimura's two-parameter model; 1000 replicates) (Tamura et al. 2011) to double-check whether they were AM fungi sequences and for phylogenetic analysis. Representative sequences in the present study were deposited in GenBank (accession numbers MZ416747-MZ416780).

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare mycorrhizal functional types, shoot biomass, and soil properties across the elevational gradient. The effects of plant species, elevation, and their interactions on AM colonization were analyzed using two-way ANOVA, and pairs of mean values were compared using paired Student *t* tests at the 5% protection level to examine differences between the *C. capillacea* and *P. annua* in AM colonization at the same altitude. $P < 0.05$ was regarded as statistically significant unless noted differently. All data were tested for normality

(Kolmogorov–Smirnov test) and homogeneity (Levene's test) before statistical analysis. A forward stepwise regression analysis was used to examine the influences of elevation, plant variables, and soil properties on total colonization in the roots of *C. capillacea*.

AM fungal richness (observed OTUs) and Hill number (based on the Shannon index) were calculated using the "diversity" function in the R package "vegan." The number of shared OTUs (between rhizosphere soil and *C. capillacea* root samples, and between *C. capillacea* and *P. annua* samples) was calculated in Bioinfo Intelligent Cloud (BIC, http://www.ehbio.com/Cloud_Platform/front/#/). Specific AM fungi OTU indicator characteristics of different sample types were found by the function "indval" from the "labdsv" package in R (Dufrene and Legendre 1997). The relative abundance (RA) of an OTU refers to the ratio of its sequence number to the total sequences in each sample.

Averaged RAs of taxonomic groups were used to estimate the differential abundance of taxonomic groups between soil and *C. capillacea*, or between *C. capillacea* and *P. annua* separately (Wilcoxon rank-sum test; wilcox.test method in R; false discovery rate [FDR] < 0.05). All distance-based analyses were conducted on the respective Bray–Curtis distance matrices. A non-metric multidimensional scaling (NMDS) analysis was done in the R package "vegan," and then the "envfit" function was used to explore the relationship between AM fungi community composition and plant variables (numbers of *P. annua*, biomass of *P. annua*, numbers of total NM plants, biomass of total NM plants) and soil properties (pH, SOC, TC, TN, C/N ratio, AP). To examine the effects of soil properties, and elevation separately on AM fungi from *C. capillacea*, its rhizosphere soil, and *P. annua*, the Bray–Curtis distance matrix between pairs of samples was examined with 999 permutations using a PERMANOVA model ("Adonis" function in R package vegan). Prior to analyses, significant collinearity between environmental variables TN and SOC from Pearson correlations ($r < -0.7$ and $r > 0.7$, $P < 0.01$) resulted in those variables being grouped as Group SOC in the model. Within the group, only the variable with the highest R^2 in univariate analyses was selected in the model. Additionally, the effects of sample type and elevation were assessed with PERMANOVA separately on AM fungi in *C. capillacea* and its rhizosphere soil as well as *C. capillacea* and *P. annua* samples. Partial correlations were conducted to analyze the relationships between AM fungal colonization and diversity. Adjusted P values of correlation analyses based on the Hochberg method was performed by the "p.adjust" function in R. A PERMANOVA was used to examine the relationship between AM fungal community composition and colonization of roots of *C. capillacea* and *P. annua*.

Results

Environmental variables and predictors of AM colonization

The numbers of *C. capillacea* and *P. annua* (PAN) ranged from 11.13 to 69.93% and 4.00 to 50.62% of total plants, respectively. The ratios of *C. capillacea* and *P. annua* biomass (PAB) ranged from 2.63 to 62.40% and 0.82 to 20.50% of total plants, respectively. The numbers of total NM plants (NMN, including *C. capillacea* and other NM plants) accounted for 25.00–96.00% of the total plants, and the biomass of total NM plants (NMB) accounted for 3.78–96.45% of total shoot biomass. Elevation had significant effects on TN ($F_{3,8} = 5.41$, $P = 0.025$), NMN ($F_{3,8} = 54.85$, $P < 0.001$), NMB ($F_{3,8} = 19.43$, $P < 0.001$), PAN ($F_{3,8} = 23.45$, $P < 0.001$), and above-ground biomass ($F_{3,8} = 5.03$, $P = 0.03$) but had non-significant effects on soil pH, SOC, TC, TC:TN, AP, and PAB (Table S2).

The AM colonization rate of *C. capillacea* ranged from 2.89 to 18.84% (hyphal), 0 to 3.33% (vesicle), 0 to 0.71% (arbuscule), and 2.89 to 22.40% (total colonization). AM colonization rate in *P. annua* ranged from 8.63 to 38.20% (hyphal), 0 to 9.64% (vesicle), 0 to 1.76% (arbuscule), and 9.65 to 49.51% (total colonization). In most cases, the AM colonization of *C. capillacea* was significantly lower than that of neighboring *P. annua* at the same altitude, especially the abundance of hyphae and total colonization rate (Figs. 1 and S2). However, the abundance of arbuscules at 3904, 4285, and 4510 m and vesicles at 4510 m between roots of *C. capillacea* and *P. annua* had no statistical difference (Fig. S2). Elevation and plant species had significant negative effects on hyphal colonization ($F_{3,16} = 202.18$, $P < 0.001$; $F_{1,16} = 405.10$, $P < 0.001$, respectively), vesicle colonization ($F_{3,16} = 197.67$, $P < 0.001$; $F_{1,16} = 126.24$, $P < 0.001$, respectively), arbuscule colonization ($F_{3,16} = 46.60$, $P < 0.001$; $F_{1,16} = 55.54$, $P < 0.001$, respectively), and total colonization ($F_{3,16} = 251.63$, $P < 0.001$; $F_{1,16} = 415.32$, $P < 0.001$, respectively). A stepwise regression analysis showed total colonization of *C. capillacea* was negatively affected by elevation, followed by NMB (Total = 62.82–0.012 Elevation-0.09NMB, $P < 0.001$).

AM fungi diversity and community phylogenetic structure

After quality filtering, 12 soil and 24 root samples were clustered in 100 AM fungi OTUs that reflected 79 AM fungi OTUs in rhizosphere soil of *C. capillacea*, 65 OTUs in roots of *C. capillacea*, and 84 OTUs in roots of *P. annua* (Figs. S3a and S4). The paired Student *t* test showed AM

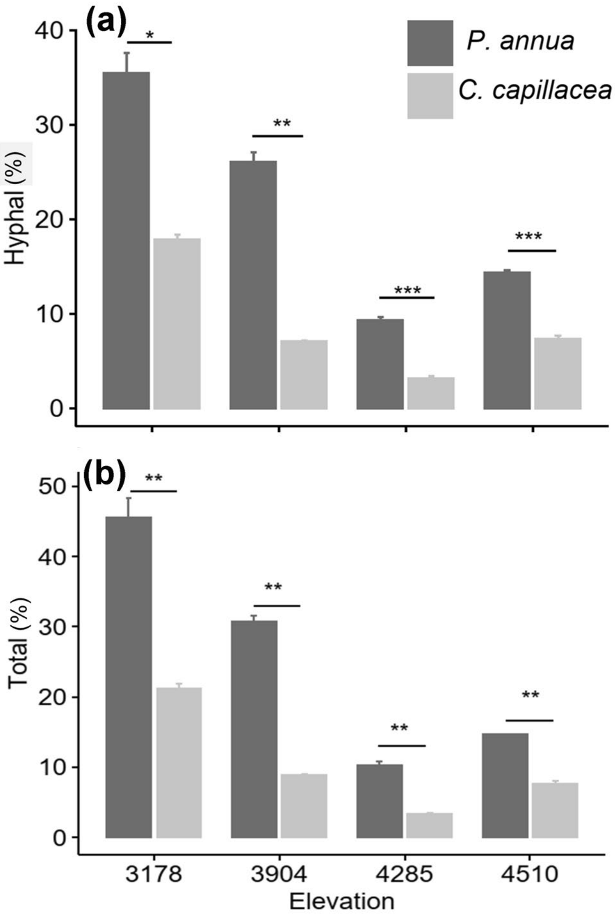

Fig. 1 Colonization (**a** hyphal; **b** total colonization) of AM fungi in roots of *Carex capillacea* and *Poa annua* along the elevational gradient. All the data are presented as mean ± SE, $n = 3$. Significant differences between the two plant species in AM colonization at same altitude by paired Student *t* tests are shown with asterisks. *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$

fungal richness in roots of *P. annua* was greater than in *C. capillacea* and soil samples at 4510 m (Fig. S3a). Conversely, the OTU richness and the Hill number from *C. capillacea* and *P. annua* were considerably lower than in the soil at 3904 m (Fig. S3). Two-way ANOVAs showed elevation gradients and sample types had significant effects on both OTU richness ($F_{3,24} = 11.71$, $P < 0.001$; $F_{2,24} = 8.62$, $P = 0.002$, respectively) and Hill number ($F_{3,24} = 4.97$, $P = 0.008$; $F_{2,24} = 3.65$, $P = 0.041$, respectively) of AM fungi. The interaction between elevation gradients and sample types had significant effect on Richness ($F_{6,24} = 3.67$, $P = 0.01$) but had little effect on Hill number. Additionally, partial correlation analysis demonstrated AM fungal diversity was not associated with colonization rate (Table S3).

The numbers of shared OTUs between rhizosphere soil and *C. capillacea* roots, as well as *C. capillacea* and *P. annua*, were 53 and 58, respectively (Fig. S4). The Dufrêne-Legendre indicator species analysis showed that only one OTU (Glomeraceae) was the indicator of AM fungus presence in *C. capillacea*. This OTU indicator value for *P. annua* was higher than that in the rhizosphere soil of *C. capillacea* (Table S4). OTUs described as indicators of *P. annua* always existed in *C. capillacea*, whereas the indicator species present in soil was rarely colonized in *C. capillacea* roots (Table S4).

AM fungi sequences from the families Claroideoglomeraceae, Glomeraceae, Acaulosporaceae, Paraglomeraceae, Ambisporaceae, and Archaeosporaceae were amplified from roots of *C. capillacea* (Fig. S5). These families were also found in *P. annua* roots and rhizosphere soil of *C. capillacea,* except that Archaeosporaceae was not found in rhizosphere soil (Fig. S5). Glomeraceae was the most abundant family in *C. capillacea* at all elevations and in *P. annua* at the highest elevation, but Claroideoglomeraceae was the most abundant family in the soil at 3178 and 4285 m, as well as *P. annua* at 3178, 3904, and 4285 m (Fig. S5). Compared with rhizosphere soil samples, AM fungi communities in roots of *C. capillacea* revealed a significant increase in RA for taxa belonging to the family Achaeosporaceae (Wilcoxon rank-sum test, FDR < 0.05; Fig. 2). The rest of the families had no significant differences for RA between soil and *C. capillacea* or between *P. annua* and *C. capillacea* (Fig. 2). The NMDS analysis based on Bray–Curtis distances revealed more pronounced differences between the roots of *C. capillacea* and its rhizosphere soil than between the roots of *C. capillacea* and *P. annua* (Fig. 3).

Predictors of AM fungal community composition

Differences in AM fungal community composition were mainly attributed to elevation, PAN and PAB (Fig. 3). Still, unlike the results for rhizosphere soil and roots of *P. annua*, elevation and soil properties had non-significant effects on AM fungi community assemblages in *C. capillacea* (Table 1). Similarly, the numbers and biomass of mycotrophic plants had non-significant effects on the AM fungi community in roots of *C. capillacea* (Table 1). In addition, a significant effect of sample type was found between soil and *C. capillacea*, yet a non-significant effect was found for *C. capillacea* and *P. annua* (Table 2). A PERANOVA showed that the abundance of hyphae had a noticeable effect on AM fungi community composition in roots of *P. annua* but had little effect on AM fungi community composition in the root of *C. capillacea* (Table S5).

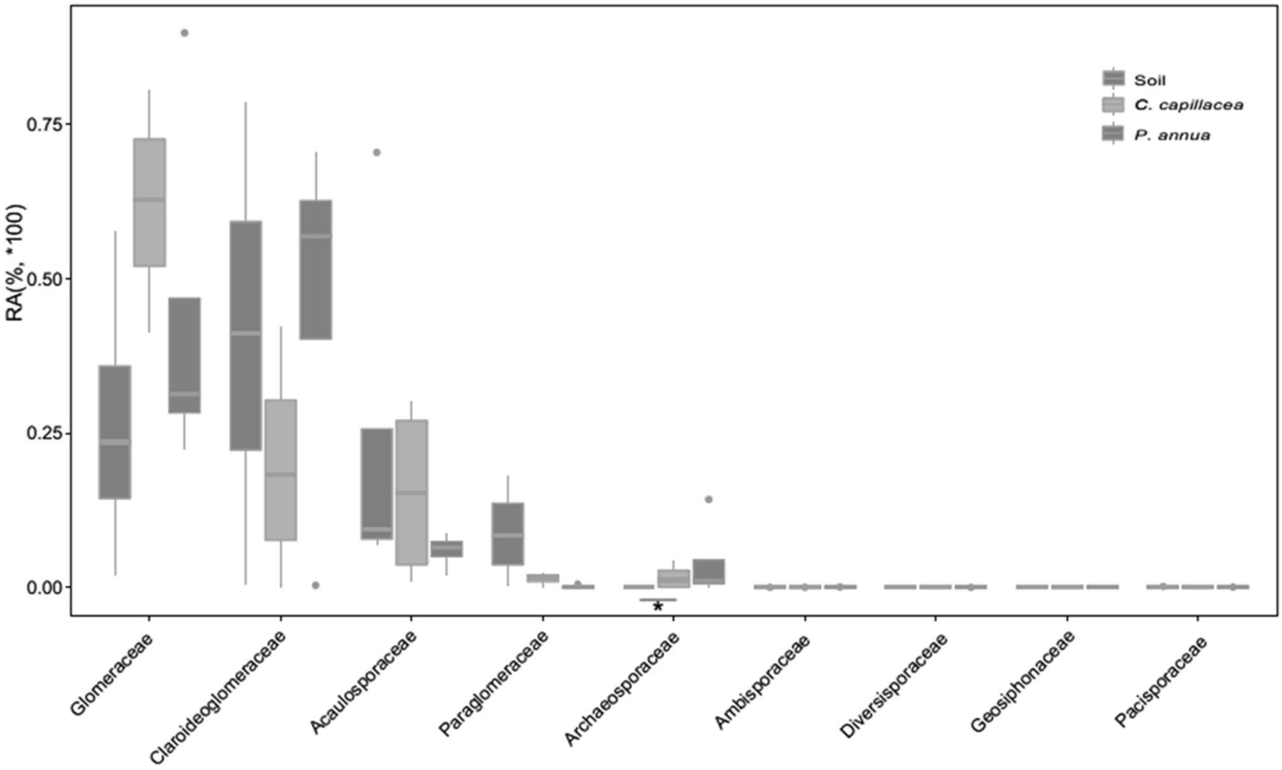

Fig. 2 Comparison of AM fungus family relative abundances between sample types (rhizosphere soil versus and *Carex capillacea* roots, or *Carex capillacea* versus *Poa annua* roots). Significant differences are marked with an asterisk (Wilcoxon rank-sum test: FDR < 0.05)

Discussion

AM colonization of C. capillacea

In agreement with our hypothesis, substantially less AM colonization (i.e., hyphae, vesicles, and arbuscles) in *C. capillacea* compared with neighboring *P. annua*. Previous studies also reported that AM colonization in other NM plant species was lower than in neighboring mycotrophic plants (Lekberg et al. 2015; Cosme et al. 2018; Wang et al. 2021). Research of the same genus as *C. capillacea* showed that the growing plants were not able to independently maintain an active mycelial network when not co-occurring with a mycotrophic plant (Ruotsalainen and Aikio 2004), and the colonization of field plants demonstrated that the presence of an active AM fungal network from mycorrhizal host plants was an important factor affecting the susceptibility of *C. capillacea* to AM fungi (Zhang et al. 2019). In our study, the NMB showed a negative correlation with total colonization in *C. capillacea*. Colonization remained low, especially that of arbuscules (< 1%), the main sites for nutrient exchange in AM symbiosis (Manjarrez et al. 2008), indicating that the colonization encountered in roots of *C. capillacea* was likely vestigial and passive. Nevertheless, we have no direct empirical evidence that colonization lacked physiological functionality.

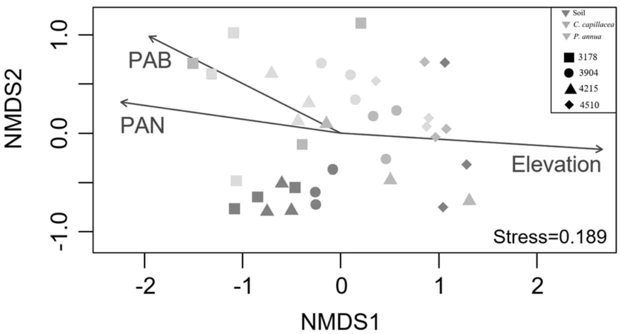

Fig. 3 Non-metric multidimensional scaling (NMDS) plot of AM fungi community composition (based on Bray–Curtis distances) and the vectors of significant environmental variables ($p < 0.05$) across sites. Arrows indicate the correlation of the two NMDS axes with the ratio of *Poa annua* biomass to total plant biomass (PAB), ratio of *Poa annua* number to total plant number (PAN) and elevation

In addition, we found AM colonization became little or none under increased altitude where always accompanied by exacerbated severe environmental conditions. This result is accordant with most studies on elevational patterns of AM fungal colonization (Wahl and Spiegelberger, 2016;

Table 1 PERMANOVA partitioning of AM fungi community assemblages decomposed by three types based on OTUs distance matrices

Parameters	df	Soil					*Carex capillacea*					*Poa annua*				
		SS	MS	Pseudo-*F*	R^2	*P*	SS	MS	Pseudo-*F*	R^2	*P*	SS	MS	Pseudo-*F*	R^2	*P*
pH	1	0.321	0.321	1.815	0.093	0.086	0.271	0.271	0.823	0.083	0.581	0.382	0.382	2.091	0.108	**0.035**
AP	1	0.464	0.464	2.623	0.134	**0.008**	0.208	0.208	0.632	0.064	0.772	0.472	0.472	2.583	0.133	0.01
Group SOC	1	0.329	0.329	1.858	0.095	0.077	0.426	0.426	1.294	0.13	0.266	0.464	0.464	2.543	0.131	**0.037**
TC:TN	1	0.196	0.196	1.107	0.057	0.353	0.195	0.195	0.592	0.06	0.766	0.25	0.25	1.371	0.071	0.256
Elevation	1	0.756	0.756	4.268	0.218	**<0.001**	0.527	0.527	1.603	0.162	0.173	0.714	0.714	3.912	0.202	**0.002**
PAN	1	0.34	0.34	1.921	0.098	0.061	0.246	0.246	0.747	0.075	0.663	0.415	0.415	2.274	0.118	**0.024**
PAB	1	0.347	0.347	1.96	0.1	0.071	0.075	0.075	0.227	0.023	0.984	0.105	0.105	0.575	0.03	0.831
Residuals	4	0.708	0.177		0.205		1.315	0.329		0.403		0.73	0.183		0.207	
Total	11	3.462			1		3.262			1		3.533			1	

Significant relationships were shown in bold. *SS*, sums of squares; *MS*, mean squares; *AP*, available phosphorus; *SOC*, soil organic carbon; *TC:TN*, total carbon:total nitrogen; *PAN*, ratio of *Poa annua* (number); *PAB*, ratio of *Poa annua* (biomass). Significant collinearity between TN and SOC was grouped into Group SOC in the model

Yang et al. (2017). This may be because it is difficult for AM fungi extraradical hyphae to survive under the adverse environmental conditions of the highest elevation, thereby constraining extension of the mycelial network linking mycotrophic and non-mycotrophic members of the plant community.

AM fungal diversity and community composition

Although the total number of OTUs in *C. capillacea* was less than that in *P. annua* and rhizosphere soil, the AM fungi diversity was similar in roots of *C. capillacea* and *P. annua* other than richness at the highest elevation. Therefore our hypothesis that AM fungi diversity in roots of *C. capillacea* would be lower than in co-occurring *P. annua* was not supported. This is accordant with the study of Wang et al. (2021) in two wetlands, which found AM fungi richness and Hill number from mycotrophic and non-mycotrophic plant species did not differ significantly. In addition, our study showed AM fungal diversity in *C. capillacea* and *P. annua* roots also may not be correlated with colonization intensity for *C. capillacea* or *P. annua*. These findings suggest that the interaction of host and non-host with AM fungi is more nuanced than the dichotomy indicates.

Like most studies, we observed that AM fungi richness between *C. capillacea* and rhizosphere soil had apparent differences at low altitude, with lower values in roots relative to rhizosphere soil, indicating the selection of a reduced number of fungal taxa entering the plant roots (Almario et al. 2017). Interestingly, the number of shared OTUs between rhizosphere soil and *C. capillacea* roots was less than that between *C. capillacea* and *P. annua*. Additionally, all AM fungi indicator species in soil were rarely found in *C. capillacea* roots located millimeters apart. Because AM fungi have no significant saprotrophic abilities, these results likely are explained as biological consequences when considering the differences for AM fungal growth within roots versus in soil tightly surrounding roots (Brundrett 2002; Gao et al. 2019). In other words, the "habitat" of AM fungi may predominantly reflect its intraradical portion. Previous studies also have found that the surrounding mycelium network of not all AM fungi has the ability to cross the selection filters imposed by non-mycorrhizal plants (Cosme et al. 2018).

AM fungal community discrimination between *C. capillacea* and *P. annua* was not detected along elevational gradients. It is possible that colonization of the roots of NM species could position AM fungi advantageously for recovery and retention of mineral nutrients when those roots of the NM species die. Therefore, the root-colonized AM fungi in *C. capillacea* were most likely a subset of those in neighboring mycotrophic plants (including but not limited to *P. annua*). Thus, the hypothesis that these AM fungi are derived from extensions of host plants' mycelium networks

Table 2 Summary of the results of PERMANOVA of AM fungi community structures

Source	df	SS	MS	Pseudo-*F*	R^2	*P*
Soil & *Carex capillacea*						
Sample type	1	0.949	0.949	4.228	0.124	**0.002**
Elevation	3	2.533	0.844	3.762	0.33	**< 0.001**
Sample type: Elevation	3	0.601	0.2	0.892	0.078	0.577
Residuals	16	3.591	0.224		0.468	
Total	23	7.673			1	
Carex capillacea & *Poa annua*						
Sample type	1	0.336	0.336	1.497	0.047	0.153
Elevation	3	2.464	0.821	3.654	0.345	**< 0.001**
Sample type: Elevation	3	0.735	0.245	1.091	0.103	0.353
Residuals	16	3.596	0.225		0.504	
Total	23	7.131			1	

Significant relationships were shown in bold. *SS*, sums of squares; *MS*, mean squares

is supported again. Even so, the result of comparison of AM fungi diversity and community composition in the roots of *C. capillacea* and other sample types suggests that a potential source of AM fungi was previously mostly underestimated and subsequent studies should include putative NM plants for a complete understanding of AM symbionts.

AM fungi can exchange of P for C using host plants as resource islands (Lekberg et al. 2010). In addition, when the mycorrhizal network connects both non-photosynthetic plants and green plants, the non-photosynthetic plants can obtain carbon via the mycorrhizal fungi associated with their roots (Gomes et al. 2017). These myco-heterotrophic plants are preponderantly colonized by the genera *Rhizophagus* and *Funeliforms*, both belonging to the family Glomeraceae (Merckx et al. 2012). Perhaps co-incidentally, our study showed Glomeraceae was the most abundant family in the roots of *C. capillacea*. This may be partially related to Glomeraceae being a highly species-rich family with a lot of apparently ruderal species. Moreover, the genera (e.g., *Rhizophagus*, which was abundant in our study) from this family might mediate inter-plant C transfer (Bidartondo et al. 2002). As obligate symbionts AM fungi are highly dependent on plant-derived C, and the connection of a mycelium network between a host and non-host may allow the fungi to obtain carbon from host plants and store and protect it inside NM neighbor roots (Lekberg et al. 2015).

Implications for environmental impact assessment

Numerous biotic and abiotic factors vary along an elevation gradient, including the proportion of mycorrhizal plants, soil physical and chemical properties, temperature, moisture, and so on; thus, it is an ideal site to investigate AM community patterns. Our PERMANOVA results indicated that elevation and soil properties had weak effects on AM fungi community assemblages in *C. capillacea*. Therefore, our hypothesis that the community composition in *C. capillacea* along the altitude gradient was mainly affected by alpine environmental conditions and to a lesser extent by neighboring host plants was not supported. Still, elevation and plant variables (numbers and biomass of *P. annua*) had significant influences on the entire AM fungal community along the elevational gradient. Moreover, sample type had no significant influence on AM fungi community assemblages between *C. capillacea* and *P. annua*, again supporting our inference that they had the same source, mostly *P. annua*.

Nonetheless, the AM fungal community composition was largely unexplained by the factors we assessed, especially with respect to affecting AM fungi community assemblages in *C. capillacea*, which further indicates that the effects of other factors on AM fungal community composition were important. For example, Guo et al. (2020) demonstrated that plant communities had more significant effects on the AM fungi community than soil properties. Future studies should determine whether the environmental factors (including plant properties) that affect AM fungi community assembly and adaptive differentiation at large spatial scales are different from those acting along elevational gradients.

Conclusions

We found that AM fungi colonization in *C. capillacea* was lower than that in *P. annua*. The existence of NM plants and the variations of elevation were important factors affecting the AM fungi colonization of *C. capillacea*. Unlike AM fungi colonization, diversity and community composition in the roots of *C. capillacea* was similar to that of *P. annua*, and the communities were most likely a subset of those in the roots of *P. annua*. Furthermore, differences in elevation, numbers, and biomass of *P. annua* were important for the whole AM community assemblages with respect to divergence along the elevational gradient. The factors that affect AM community assemblages in *C. capillacea* roots, however, are still unclear. This is the first direct description of AM fungal diversity, community composition, and influencing factors in an NM plant species along an elevation gradient which reveals the ecological pattern in AM fungi diversity.

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Author contribution ZB. Jin and JP. Gai designed the research; HB. Zhang and NN. Mi collected the samples; HB. Zhang, YB. Chai, and HJ. Xie performed research; HB. Zhang and Y. Yang analyzed data; Y. Yang wrote the draft of the manuscript; all the authors reviewed and edited the final manuscript; and ZB. Jin, JP. Gai, and XL. Li supervised the research.

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Declarations

Competing interests The authors declare no competing interests.

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