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Rhizosphere and root fungal community of the invasive plant *Galinsoga quadriradiata* changes along its elevational expansion route

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Abstract

Fungal communities related to invasive plants may change with an elevational gradient, which may affect the performance and invasiveness of invasive plants. Our recent study revealed that root arbuscular mycorrhizal fungi colonization rate of invasive plant *Galinsoga quadriradiata* decreased with elevation. However, it is unclear whether it is caused by the changes in the fungal community along elevation. To address this issue, we used high-throughput sequencing techniques, functional groupings and linear statistics to examine how fungal communities in the rhizosphere and roots of *G. quadriradiata* are changed across the elevation in Qinling and Bashan Mountains, China. Our results revealed that species diversity and composition of the rhizosphere and root fungal communities changed along the elevation. The Shannon–Wiener diversity index in the rhizosphere and roots increased and decreased with elevation, respectively. In contrast, the relative abundance of pathotroph in the rhizosphere decreased while it increased in the roots with elevation. These suggest that, when the invasive plant colonizes into high altitudes, it may not suffer from limited rhizosphere fungal symbionts, but rather the ability of the plant to create and maintain these associations decreases. The invader tends to accumulate more pathogenic fungi in the roots, while the dependence on symbiotic fungi is reduced during expansion into higher elevations. These results highlight that the interactions between invasive plants and fungal community substantially change along elevation, and that belowground interactions may be key in our understanding of how invasive plants derive success in stressful, high-elevation environments.

Keywords invasive plants, range expansion, fungal diversity, fungal abundance, symbionts, pathotrophs

入侵植物粗毛牛膝菊根际土壤和根系真菌群落会沿海拔扩散路径发生变化

摘要：与入侵植物相关的真菌群落可能会随着海拔发生变化，这可能会影响入侵植物的表现和入侵力。我们最近的研究表明，入侵植物粗毛牛膝菊(*Galinsoga quadriradiata*)的根系AMF侵染率随着海拔的升高

而下降。然而，尚不清楚这是否与真菌群落沿海拔的变化相关。为此，我们采用高通量测序技术、功能分组和线性分析等方法，研究了秦巴山区的粗毛牛膝菊根际土壤和根系真菌群落组成与结构沿海拔的变化趋势。研究结果表明，粗毛牛膝菊根际土壤和根系真菌群落物种多样性和种类组成随海拔升高发生了显著变化。在根际土壤中真菌群落香农–威纳多样性指数随着海拔升高呈上升趋势，而在根系中则呈下降趋势。同时，在根际土壤中共生真菌的相对丰度随海拔高度没有发生显著变化，而在根系中呈下降趋势；在根际土壤中病原真菌的相对丰度随着海拔呈下降趋势，而在根系中呈上升趋势。这些研究结果暗示，该种在向研究区域高海拔地区入侵时，土壤共生真菌的供给并不会缺乏，但其与这些真菌之间建立并维持共生关系的能力降低了；在向高海拔扩张过程中，该种倾向于在根系中积累更多的病原真菌，而减少对共生真菌的依赖。这些研究结果说明，入侵植物与真菌群落之间的相互作用会沿着海拔梯度发生显著变化，植物地下部分与真菌的相互作用可能是我们理解外来种如何在高海拔环境中入侵成功的关键。

关键词：入侵植物，范围扩张，真菌多样性，真菌丰度，共生菌，病原菌

INTRODUCTION

Plant invasion is an important environmental problem because it can affect the function and stability of natural ecosystems (Feng *et al.* 2022; Liu *et al.* 2017; Vetter *et al.* 2020; Feng *et al.* 2022), and increasing evidence shows that invasive plants can spread into high-elevation mountain ranges (Pauchard *et al.* 2009). The climatic parameters and soil properties of mountain regions change dramatically along altitudinal gradients, especially with respect to temperature, precipitation and soil properties (Coutinho *et al.* 2015). These variations strongly influence the composition and evolution of plant and soil microbial communities (Coutinho *et al.* 2015). In response, many plants in these environments, and particularly invasive species, have developed numerous adaptation strategies to face the harsh conditions resulting from the increase in altitude. These adaptation strategies include high cold resistance and affinity to moist soil (Petitpierre *et al.* 2016), changed life-history traits (Ansari and Daehler 2010) and enhanced tolerance to high UV-B radiation levels (Alexander *et al.* 2009; Watermann *et al.* 2019).

Furthermore, in addition to the above traits, invasive plant species also likely benefit from positive interactions with soil microbial communities. Many plants are strongly affected by interactions with arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal (ECM) fungi, which can play important roles in the invasion of exotic plants (Aslani *et al.* 2019; Kong *et al.* 2022; Rodriguez-Caballero *et al.* 2020; Smith and Read 2008; Thorn 2003; van der Putten *et al.* 2007a). For example, the association with mycorrhizal fungi can facilitate the expansion of invasive plants by promoting their growth relative to those of non-mycorrhizal plants (Hayward *et al.* 2015; Menzel *et al.* 2017). However, these no differ between native and non-native lineage of *Phragmites* root fungal and bacterial community diversity (Bickford *et al.* 2018). Therefore, the effect of soil and root microorganisms on alien plant invasions is still not well understood. At the same time, invasive plants can alter the diversity and species composition of soil fungal communities (Gaggini *et al.* 2018; Řezáčová *et al.* 2021). For example, invasion of *Centaurea solstitialis* and *Aegilops triuncialis* in California grasslands has been documented to alter soil microbial composition (Batten *et al.* 2006) and another study found that invasive *Triadica sebifera* can alter soil AMF communities (e.g. colonization and spore germination) via root exudates (Tian *et al.* 2021).

In mountain ranges, soil fungal communities have been shown to shift dramatically with elevation due to changes in environmental drivers (Jarvis *et al.* 2015; Siles *et al.* 2017). Generally speaking, soil fungal diversity decreases as elevation increases, likely because of changes in soil pH and mean annual temperature (Bahram *et al.* 2012; Ping *et al.* 2017). However, other contradictory results have also been reported. For example, Siles and Margesin (2016) found that soil fungal community relative size, fungal abundance and microbial activity increased with elevation. Furthermore, relative contribution of different soil fungal functional guilds (e.g. symbiotes, saprotrophs and pathogens) also change with elevation in mountain ranges (Veach *et al.* 2018). The richness of operational taxonomic units (OTUs) of soil AMF,

which are generally symbiotic with plants, usually decreases with elevation (Geml 2017; Pellissier *et al.* 2013; Vašutová *et al.* 2017), whereas saprotroph abundance increases with elevation (Eduardo *et al.* 2018) and fungal pathogen abundance depends on the elevational distribution of the host species (Merges *et al.* 2018). These different changes in abundance may impact the expansion of invasive plants. Previous studies suggest that soil AMF community could co-disperse with non-native plants across elevational gradients (Clavel *et al.* 2021), and invasive plants subsequently could obtain more advantages than native competitors in higher altitudes (Urcelay *et al.* 2019). However, these functional group effects as they relate to plant invasions, and particularly as they relate to invasions in mountain ranges, are still far from being resolved and merit further consideration.

Plant invasion can exert important effects on the soil fungal community, but the latter can also affect the invader via plant–soil feedbacks (de Souza *et al.* 2018; Si *et al.* 2013). One potential way to gain insight into the magnitude and direction of these effects is to compare the properties and characteristics of soil mycorrhizal communities and those communities specifically associated with plant roots (Lumibao *et al.* 2020). Specifically, rhizosphere fungal abundance and diversity can inform whether invasive plants have access to different fungal species and functional groups, but measuring these characteristics for root samples will provide further confirmation as to whether the plants are actually associating with them. Although this approach is not novel, it has primarily been limited to use in agricultural studies investigating AMF associations of crop species (Mirás-Avalos *et al.* 2011; Xu *et al.* 2012), and only relatively recently has it been applied within a broader ecological framework to natural systems (Lumibao *et al.* 2020). Previous work has suggested that fungal abundance (e.g. pathogenic and saprophytic) in soil can be greater than that of root samples for invasive plant species (Phillips *et al.* 2019). Although unexplored, it is also possible that the differences of fungal diversity could affect the process of invasion into mountain ranges as well. Our latest study revealed that root AMF colonization rate of *Galinsoga quadriradiata* decreased significantly with elevation in Chinese mountain ranges, and that higher-altitude populations maintained a lower root AMF colonization rate than the lower-altitude populations, even in a common garden (Liu *et al.* 2021a), lending support to that local fungal community may impact the dispersal of invasive plant. However, it is still unclear whether this is caused by the changes of the fungal community along elevation or by population differences.

In this study, we aimed to examine how the rhizosphere and root fungal community of populations of invasive *G. quadriradiata* changes with elevation in Chinese mountain ranges. Our previous studies suggest that this species could become more aggressive and expand into high altitudes in the future with greater dispersal traits (Liu *et al.* 2018, 2021b; Yang *et al.* 2018) and perhaps as a consequence of its ability to form symbiotic associations with AMF during its expansion into mountain ranges (Liu *et al.* 2021a). This, in turn, increased its competitive ability and promoted its expansion in areas with available fungal symbionts (unpublished data). To explore this, we measured fungal community composition in the rhizosphere and roots of *G. quadriradiata* along an elevational gradient in the Qinling and Bashan Mountains in central and southwestern China. We asked three questions: (i) How do rhizosphere and root fungal community compositions of *G. quadriradiata* change across elevation? (ii) What are the main drivers of fungal community composition? (iii) Does root fungal community composition correlate with the rhizosphere community composition? Answering these questions will provide new information as to whether the associations between *G. quadriradiata* and soil fungi are limited by a lack of soil fungal inoculum during the expansion, and further inform practitioners and land managers about the potential for this invader to continue to invade along elevational gradients.

MATERIALS AND METHODS

Study species

Galinsoga quadriradiata (Asterales: Asteraceae), is an annual herb native to Central and South America and was first recorded invading China in 1978 on Lushan Mountain in Jiujiang City of Jiangxi Province (Liu *et al.* 2016, 2018, 2021a). It can cause serious ecological and economic losses for the natural and agricultural ecosystems due to its large seed yield and high population density (Kabuce and Priede 2010; Liu *et al.* 2016). It is widely distributed in several provinces, and has caused serious invasion in the Qinling and Bashan Mountains (Liu *et al.* 2016; Yang *et al.* 2018; Zhang *et al.* 2022). This invasive plant can form a close symbiotic relationship with soil fungi

such as AMF, and this symbiotic relationship has been shown to significantly differ across elevations (Liu *et al.* 2021a).

Study area

The Qinling and Bashan Mountains are important areas for the ecological protection and research, which are located in central and southwestern China (30°5′–34°59′ N, 102°54′–112°4′ E, about 222 300 km^2) (Liu *et al.* 2021b). And, the topography characters and environmental conditions of these mountains are complex and distinct between the northern and southern regions (Wang *et al.* 2017). The highest elevation of the Qinling Mountains is 3767 m a.s.l., while the highest peak of Bashan is 3105. These mountains are the watershed between the Yangtze and Yellow rivers, serve as the most important barriers between the southern and the northern regions of central China (Liu *et al.* 2021b).

The low-elevation areas on the southern slope of the Qinling Mountains have a subtropical climate; while the high-elevation areas on the southern slope and all the altitude areas on the northern slope have lower temperatures and reduced precipitation, and the climate is mainly temperate. The average annual temperature in this range is 11–13 °C and the average annual precipitation is between 590 and 764 mm (Zhao *et al.* 2014). Because it is located about 50 km south of the Qinling Mountains, the Bashan Mountains have a subtropical climate as a whole, and the two mountains extend in parallel from east to west (Zhan *et al.* 2009). The average annual temperature of the Bashan Mountains is 14.5–16.5 °C, and average annual precipitation is between 800 and 1400 mm (Li *et al.* 1990). The Qinling and Bashan Mountains act as important natural obstacles for defending exotic species from entering northern regions of China following their introduction and establishment in the southeast.

Soil and root sample collection

In May 2017, we set up 14 sites in the Qinling and Bashan Mountains (Table 1; Fig. 1). The elevation interval between each sampling site is 300–500 m ranging from 370 to 1947 m a.s.l. At each site, we randomly selected ten individuals of *G. quadriradiata* and collected fresh root (>2 g) and rhizosphere samples (>5 g) from individual plants into cryopreserved tubes. The adhering soil and plant debris on the root were carefully removed, and then washed using water before putting into the tubes. The rhizosphere samples consisted of soil up to 1 cm away from the roots. These tubes were then immediately stored in a dry ice freezer and taken back to the laboratory and kept in a −80 °C freezer until samples could be processed. Meanwhile, we collected soil sample (approximately 1 kg) by mixing the rest of rhizosphere from each site to measure the soil chemical and physical properties. The soil samples were air-dried and finely ground before analyzing. We measured the soil chemical and physical properties based on the method described by Liu *et al.* (2021a). This included quantifications of soil total nitrogen concentrations (TN, mg g^{-1}), soil total phosphorous concentrations (TP, mg g^{-1}), soil available nitrogen (a combination of soil nitrate-nitrogen concentration [NO_3^--N, mg kg^{-1}] and soil ammonium-nitrogen concentration [NH_4^+-N, mg kg^{-1}]), soil available phosphorous (AP, mg kg^{-1}) and soil dissolved organic carbon concentration (DOC, mg kg^{-1}). All the extracted soil solutions were measured using a continuous flow analyzer (SEAL AutoAnalyzer III, Germany). Then, soil electrical conductivity (EC, μS cm^{-1}) and the pH value were, respectively, measured using EC and pH Meters (REX DDS-11C and PHS-25, INESA Scientific Instrument Co., Ltd, Shanghai, China) in a 1:2.5 (w/v) soil water suspension. We additionally measured soil water content (WaterC, %) using a portable soil moisture tachometer (HH2 Moisture Meter).

DNA extraction

The rhizosphere samples from seven sites were chosen based on the elevation for the rhizosphere fungal community analysis. Five rhizosphere samples of each site (n = 35, seven sites × five replicates) were randomly selected for DNA extracting. We mixed ten root samples from each site together for the root fungal community analysis. The mixed root samples of all sites (n = 14) were used for DNA extracting.

Genomic DNA were extracted from 0.25 g of homogenized fresh rhizosphere or root samples using the E.Z.N.A. Soil DNA Kit (Omega Bio-tek, Hibind, Germany) according to the instructions of the manufacturer. The concentration and purity of DNA were analyzed with a NanoDrop One spectrophotometer (Thermo Fisher Scientific) at a ratio of 260 nm; DNA quality was assessed by 1.8% agarose gel electrophoresis. Isolated DNA was kept frozen at −20 °C until used (Phillips *et al.* 2019; Zhang *et al.* 2018).

Library construction and sequencing

The internal transcribed spacer (ITS) 1 region located in fungal chromosomes was amplified with the primer pair ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′)

Table 1: The locations of the elevational populations of *Galinsoga quadriradiata* for the field survey

Site ID	Latitude (N°)	Longitude (E°)	Elevation (m)	Root sample ID	Soil sample ID	Mountains
1	32.48628	108.881	370	BR1	—	Bashan
2	32.31353	108.9439	436	BR2	BS1	Bashan
3	32.09437	109.256	1083	BR3	BS2	Bashan
4	32.03771	109.3047	1536	BR4	BS3	Bashan
5	32.02794	109.3201	1737	BR5	BS4	Bashan
6	32.02292	109.3358	1947	BR6	BS5	Bashan
7	33.29625	108.1904	590	QR1	—	Qinling
8	33.3485	108.3209	829	QR2	QS1	Qinling
9	33.59658	108.6383	834	QR3	—	Qinling
10	33.37503	108.3473	1000	QR4	—	Qinling
11	33.77383	108.7826	1236	QR5	—	Qinling
12	33.4272	108.4202	1307	QR6	—	Qinling
13	33.54067	108.5442	1361	QR7	QS2	Qinling
14	33.43275	108.4534	1563	QR8	—	Qinling

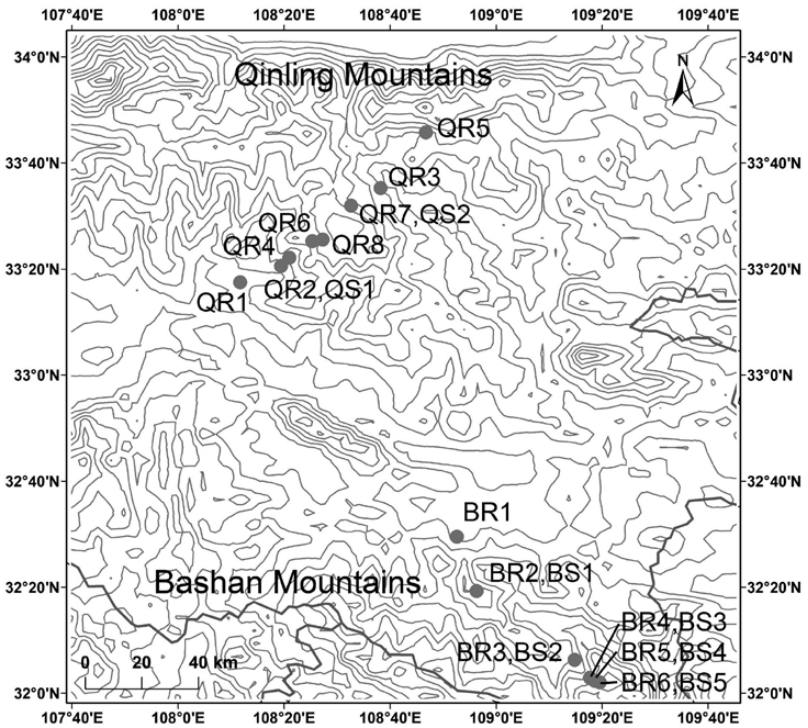

Figure 1: The map of the *Galinsoga quadriradiata* populations for the field survey.

and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) using a two-step polymerase chain reaction (PCR) (Mbareche *et al.* 2020; White *et al.* 1990). The construction of the library follows the steps described by Berry *et al.* (2011). The first PCR was performed in a final volume of 50 μL, containing template DNA 1 μL, forward primer (Vn F, 10 mmol L^{-1}) 1.5 μL, reverse primer (Vn R, 10 mmol L^{-1}) 1.5 μL, Q5 High-Fidelity DNA Polymerase (Phusion, NEB) 0.2 μL, High GC Enhancer 10 μL, Buffer 10 μL, dNTP 1 μL

and ddH_2O 24.8 μL. Thermal cycle conditions for the first ITS1 PCR consisted of an initial denaturation at 95 °C for 5 min; followed by 15 cycles of at 95 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min, with a final extension of 72 °C for 7 min, then refrigerate at 4 °C. The PCR amplification products were extracted from a 1.8% agarose gel (120 V, 40 min), purified further using a MinElute® PCR Purification Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol, and 10 μL of the purified and diluted product was used as template for indexing PCR to allow a multiplexed sequencing. The second (Solexa) PCR mixture was containing 10 μL template DNA, 1 μL forward connector (MPPI-a 10 mmol L^{-1}), 1 μL reverse connector (MPPI-b 10 mmol L^{-1}), 2× Phμsion HF MM 20 μL and 8 μL ddH_2O. The PCR conditions were: 98 °C for 30 s followed by 10 cycles of at 98 °C for 10 s, 65 °C for 30 s, 72 °C for 30 s, with a final extension of 72 °C for 5 min. DNA was recovered after 1.8% agarose gel electrophoresis of the second PCR products. Qubit 2.0 fluorescent agent was used to quantify the recovery of the products, and all samples were mixed according to the measured DNA concentration by the 1:1 ratio and fully mixed. Finally, we checked the quality of the library and paired-end sequencing was carried out on an Illumina HiSeq 2500 (Illumina Inc., San Diego, CA) with a qualified library. Qualified library was sequenced using Illumina HiSeq 2500 by Biomarker Co., Ltd (Beijing, China).

Bioinformatic analysis

We collated the original data according to the minimum overlap (10 bp) and the maximum allowable error ratio of the overlap area of 0.2 for all the reads of each sample with FLASH (Version 1.2.7) to get the raw tags. High-quality clean tags were obtained by Trimmomatic (Version 0.33) through quality filtering on the raw tags (forward and reverse) (Bolger *et al.* 2014). To obtain final effective tags, the chimeric sequences were identified and removed using UCHIME (Version 4.2) (Edgar *et al.* 2011). Sequencing reads with greater than 97% similarity threshold were clustered into OTUs using the UCLUST algorithm in Quantitative insight into the microbial ecology software (QIIME Version 1.8.0; http://qiime.org/) (Caporaso *et al.* 2010), and we made taxonomic annotations on the OTUs based on the UNITE (fungi, https://unite.ut.ee/) taxonomic database. The alpha diversity indices (ACE, ACE richness estimator; Chao1, Chao1 richness estimator; Simpson diversity index; Shannon–Wiener diversity index) were calculated based on the number of OTUs detected in the samples and were generated by Mothur software (Version v.1.30) (Schloss *et al.* 2009). QIIME software was also used to carry out the Beta diversity analysis to compare the similarity of different samples in species diversity.

OTU functional group assignment

The fungal OTUs were taxonomically parsed by ecological guilds or 'functional groups' using the online application FUNGuild (http://www.stbates.org/guilds/app.php) (Nguyen *et al.* 2016). The tool assigns guild based on the current state of knowledge and with a confidence level as follows: 'highly probable', 'probable' and 'possible' (suspected but not proven, with the conflicting reports cited). Using FUNGuild, OTUs were assigned into three functional groups (trophic mode): 'pathotroph', 'saprotroph' and 'symbiotroph' using only data with confidence levels as 'highly probable' and 'probable'. After processing OTUs through FUNGuild, we also selected AMF (Glomeromycotina) from the symbiont group to analyze the diversity and species composition of AMF specifically (Phillips *et al.* 2019).

Data analyses

We constructed a linear regression analysis to test the changes of fungal community alpha diversity, number of OTUs, number of genera and OTUs relative abundance of fungal functional groups in the rhizosphere and roots of *G. quadriradiata* along the elevation. We used the weighted algorithm of Bray–Curtis Distance to analyze the Beta diversity because it can consider both the presence and relative abundance of species. We used detrended correspondence analysis (DCA) and transformation-based redundancy analysis (tb-RDA) to analyze the relationship between fungal community composition (the phylum level) and environmental factors (WaterC, DOC, TN, TP, NH_4^+-N, NO_3^--N, AP, pH, elevation and EC). The results of DCA showed that lengths of the first four axes for both the rhizosphere and root samples were less than 3, indicating that RDA should be used to analyze it. We thus used tb-RDA to analyze the relationship between environmental factors and the fungal community composition. The statistical significance of the tb-RDA was tested using permutation tests (999 permutations; $P < 0.05$).

In addition, we used Pearson's correlation analysis to examine the relationship of the fungal community diversity (alpha diversity and number of OTUs) and relative abundance (the phylum level and functional

groups) between the rhizosphere and root samples. Only the data from the 7 sites with both root and rhizosphere soil samples were taken into the analysis. Furthermore, the same way was used to examine the relationship between fungal community diversity (the phylum level, both rhizosphere and root samples) and environmental factors, and to examine the relationship between fungal community diversity (the genus level, both rhizosphere and root samples) and elevation. All data analyses were performed in R Version 4.0.3 (R Core Team 2021). Packages *psych* (Revelle 2021), *vegan* (Dixon 2003) and *ggplot2* (Wickham 2016) were used for calculation, statistical analysis and data visualization.

RESULTS

The fungal community composition

We observed a total of 722 OTUs for the rhizosphere samples of *G. quadriradiata*. These OTUs belonged to 12 phyla, 32 classes, 64 orders, 108 families, 165 genera and 144 species. Our results showed that the Shannon–Wiener diversity index of the rhizosphere fungal communities significantly increased with elevation (R^2 = 0.12, P = 0.042, Fig. 2a) while the number of genera (R^2 = 0.05, P = 0.195, Fig. 2c) was not significantly correlated with elevation. The relative rhizosphere pathotroph OTU abundance (R^2 = 0.15, P = 0.022, Fig. 3a) was negatively correlated with elevation while that of saprotroph OTUs (R^2 = 0.17, P = 0.014, Fig. 3c) was positively correlated with the elevation. Relative rhizosphere symbiotroph OTU abundance was not significantly correlated with elevation (R^2 = 0.06, P = 0.192, Fig. 3e).

We obtained a total of 1939 OTUs from root samples of *G. quadriradiata*. These OTUs belonged to 15 phyla, 40 classes, 81 orders, 179 families, 318 genera and 360 species. The Shannon–Wiener diversity index (R^2 = 0.34, P = 0.046, Fig. 2b) and the number of genera (R^2 = 0.32, P = 0.034, Fig. 2d) were significantly negatively correlated with elevation. The relative root pathotroph OTU abundance significantly increased (R^2 = 0.55, P = 0.006, Fig. 3b) while that of symbiotrophs significantly decreased (R^2 = 0.41, P = 0.024, Fig. 3f) with elevation. Relative saprotroph OTU abundance was not significantly correlated with elevation (R^2 = 0.03, P = 0.565, Fig. 3d).

The Beta diversity of fungal and AMF communities in the rhizosphere (Supplementary Figs S1 and S2) and roots (Supplementary Figs S3 and S4) of *G. quadriradiata* differed between high- and low-elevational sites. The OTU relative abundance and species composition of rhizosphere fungal communities varied across elevation at both the phylum and family levels (Supplementary Figs S5 and S6). The OTU relative abundance of phylum Ascomycota was highest at each elevational site (Supplementary Fig. S5). Similarly, OTU relative abundance and species composition of the root fungal communities also varied across elevation at both the phylum and family levels (Supplementary Figs S7 and S8). The OTU relative abundance of phylum Ascomycota and Olpidiomycota changed most obviously with elevation (Supplementary Fig. S7). The OTU relative abundance of phylum Ascomycota first decreased and then increased with elevation, while that of phylum Olpidiomycota showed the opposite trend (Supplementary Fig. S7). At the genus level, the OTU relative abundance of most genera was significantly correlated with elevation in both the rhizosphere and roots (Supplementary Tables S3 and S4). These genera belong to phyla Ascomycota and Basidiomycota. In addition, the OTU relative abundance of AMF communities in both the rhizosphere (R^2 = 0.04, P = 0.273, Supplementary Fig. S9a) and roots (R^2 = 0.21, P = 0.101, Supplementary Fig. S9b) was not significantly correlated with the elevation.

The effects of environmental factors

Environmental factors explained 22.05% of the total changes in the rhizosphere fungal community composition at the phylum level (Fig. 4). The results of permutation tests suggested that variation in the rhizosphere fungal community composition at the phylum level were highly correlated with elevation ($F_{1,\ 23}$ = 4.326, P < 0.001), soil pH ($F_{1,\ 23}$ = 4.368, P = 0.002), NH_4^+-N ($F_{1,\ 23}$ = 3.759, P = 0.003), AP ($F_{1,\ 23}$ = 4.585, P < 0.001), DOC ($F_{1,\ 23}$ = 2.586, P = 0.024) and TN ($F_{1,\ 23}$ = 2.762, P = 0.024). The first two axes, RDA1 and RDA2, explained 11.37% and 10.68%, respectively, of the total variations of the rhizosphere fungal community composition at the phylum level. Pearson's correlation analysis revealed that the OTU relative abundance of Basidiomycota was negatively correlated with soil pH and TN (Supplementary Table S1). In contrast, the OTU relative abundance of Blastocladiomycota was positively correlated with soil pH (Supplementary Table S1). The OTU relative abundance of Glomeromycota was negatively correlated with WaterC (Supplementary Table S1). The OTU

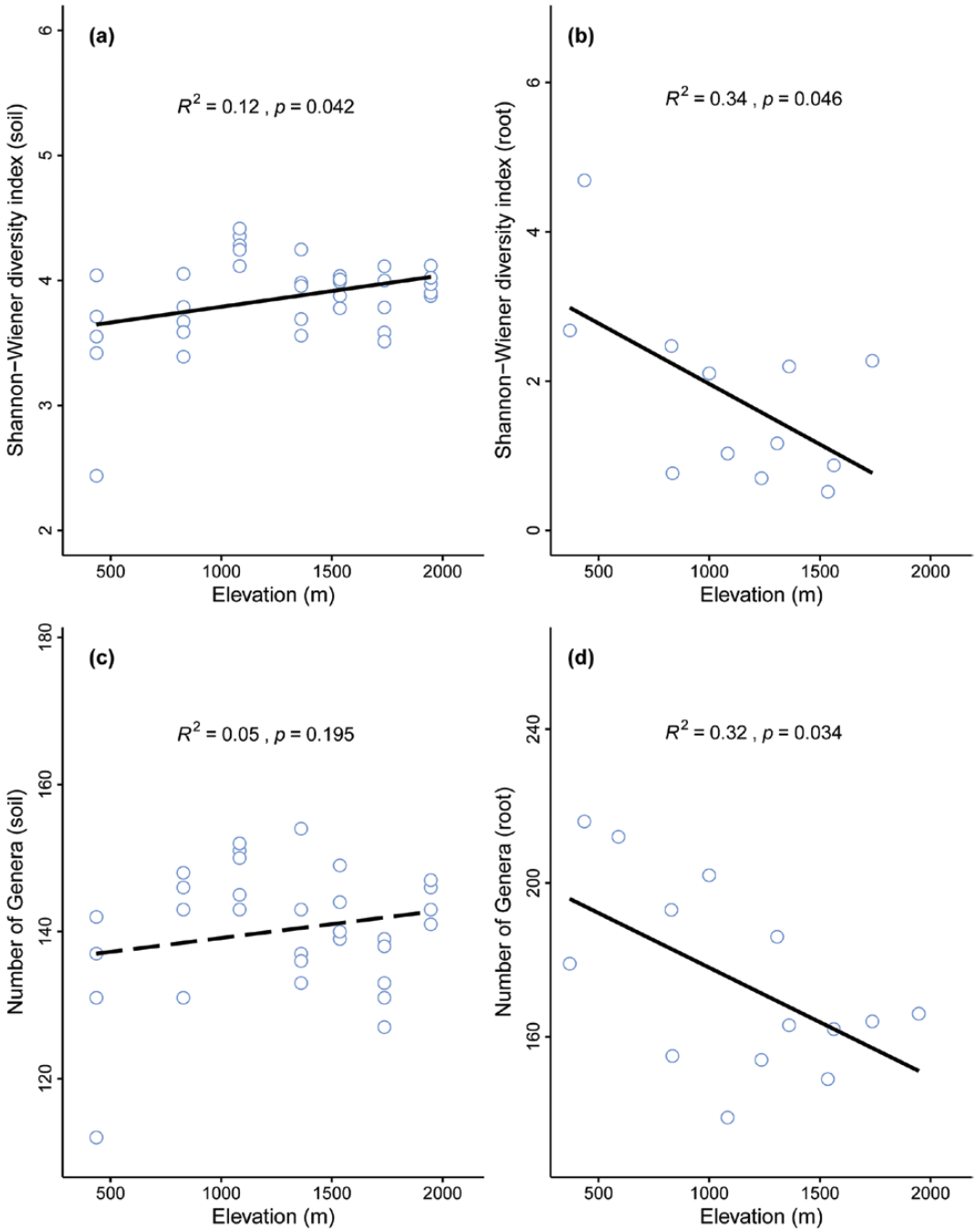

Figure 2: The linear correlations between elevation and Shannon–Wiener diversity index and the number of fungal genera in rhizosphere (**a**, **b**) and root (**c**, **d**) of *Galinsoga quadriradiata*.

relative abundance of Kickxellomycota was negatively correlated with NH_4^+-N (Supplementary Table S1). The OTU relative abundance of Neocallimastigomycota was negatively correlated with TP (Supplementary Table S1). The OTU relative abundance of Olpidiomycota was positively correlated with NH_4^+-N (Supplementary Table S1). The OTU relative abundance of Rozellomycota was positively correlated with EC, TN and TP (Supplementary Table S1). In addition, the OTU relative abundance of the pathotroph functional group was negatively related to elevation (Supplementary Table S1). The OTU relative abundance of the saprotroph functional group was significantly associated with the soil pH (negative), NH_4^+-N (positive) and elevation (positive; Supplementary Table S1). The OTU relative abundance of the symbiotroph functional group was negatively related to the soil pH (Supplementary Table S1).

The environmental variables explained 55.55% of the total changes in the root fungal community composition of *G. quadriradiata* at the phylum level

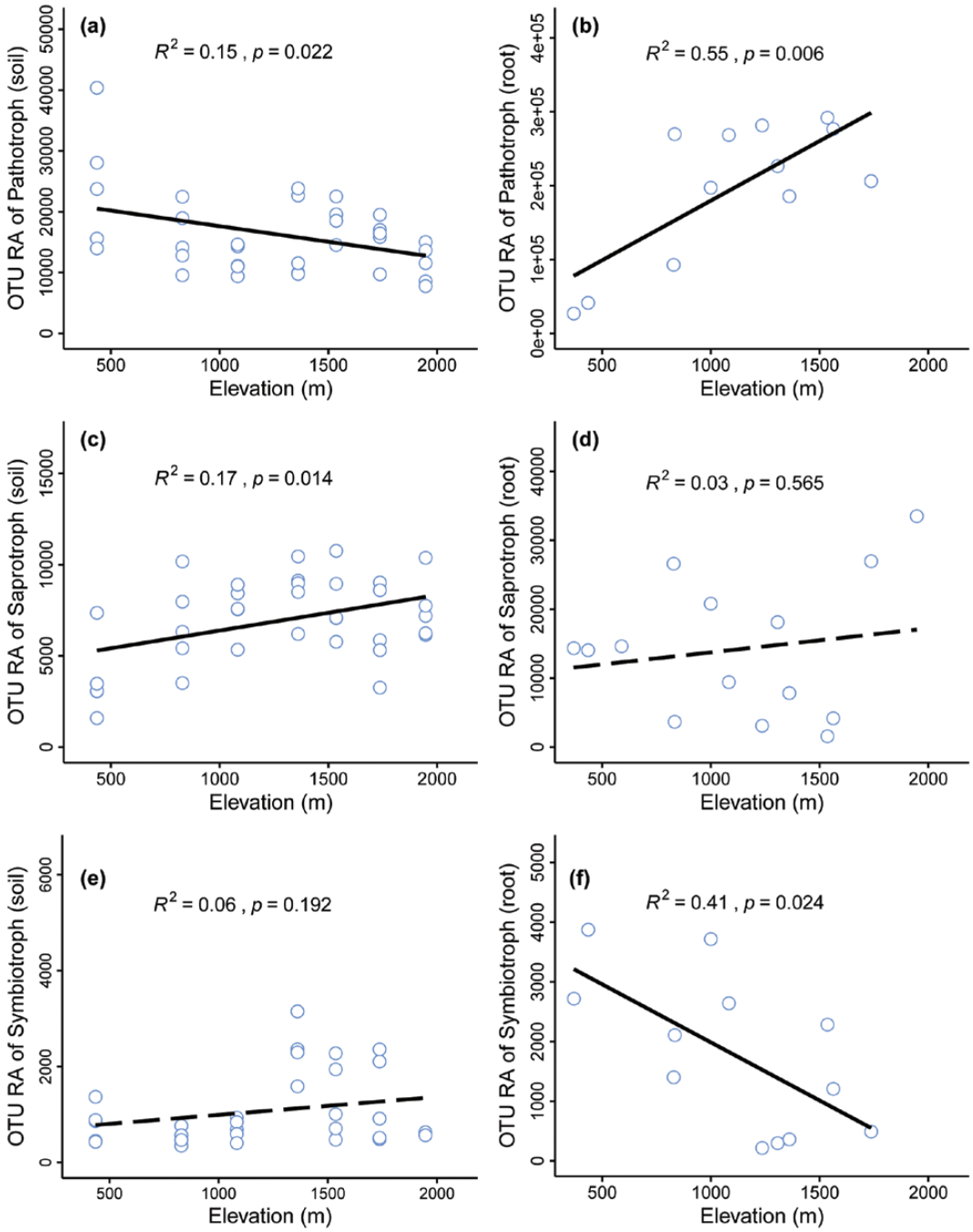

Figure 3: The linear correlations between elevation and OTU relative abundance (OTU RA) of pathotroph, saprotroph and symbiotroph in rhizosphere (**a**, **c**, **e**) and root (**b**, **d**, **f**) of *Galinsoga quadriradiata*.

(Fig. 5). The results of permutation tests suggested that variation in the root fungal community composition at the phylum level was closely related to DOC ($F_{1,3}$ = 10.820, P = 0.006) and soil pH ($F_{1,3}$ = 4.407, P = 0.050). The RDA1 and RDA2 explained 48.38% and 7.17%, respectively, of the total variation. Pearson's correlation analysis revealed that the OTU relative abundance of Aphelidiomycota was closely correlated to EC and NH_4^+-N, while that of Blastocladiomycota was negatively correlated with soil pH, NH_4^+-N, elevation and TN (Supplementary Table S2). The OTU relative abundance of Kickxellomycota was associated with TP, while the OTU relative abundance of Mucoromycota was correlated with soil pH (Supplementary Table S2). The OTU relative abundance of Neocallimastigomycota and Entomophthoromycota was closely correlated to TN (Supplementary Table S2). The OTU relative abundance of Rozellomycota was highly related to NO_3^--N and AP (Supplementary Table S2). In addition, the OTU relative abundance of the pathotroph functional group was

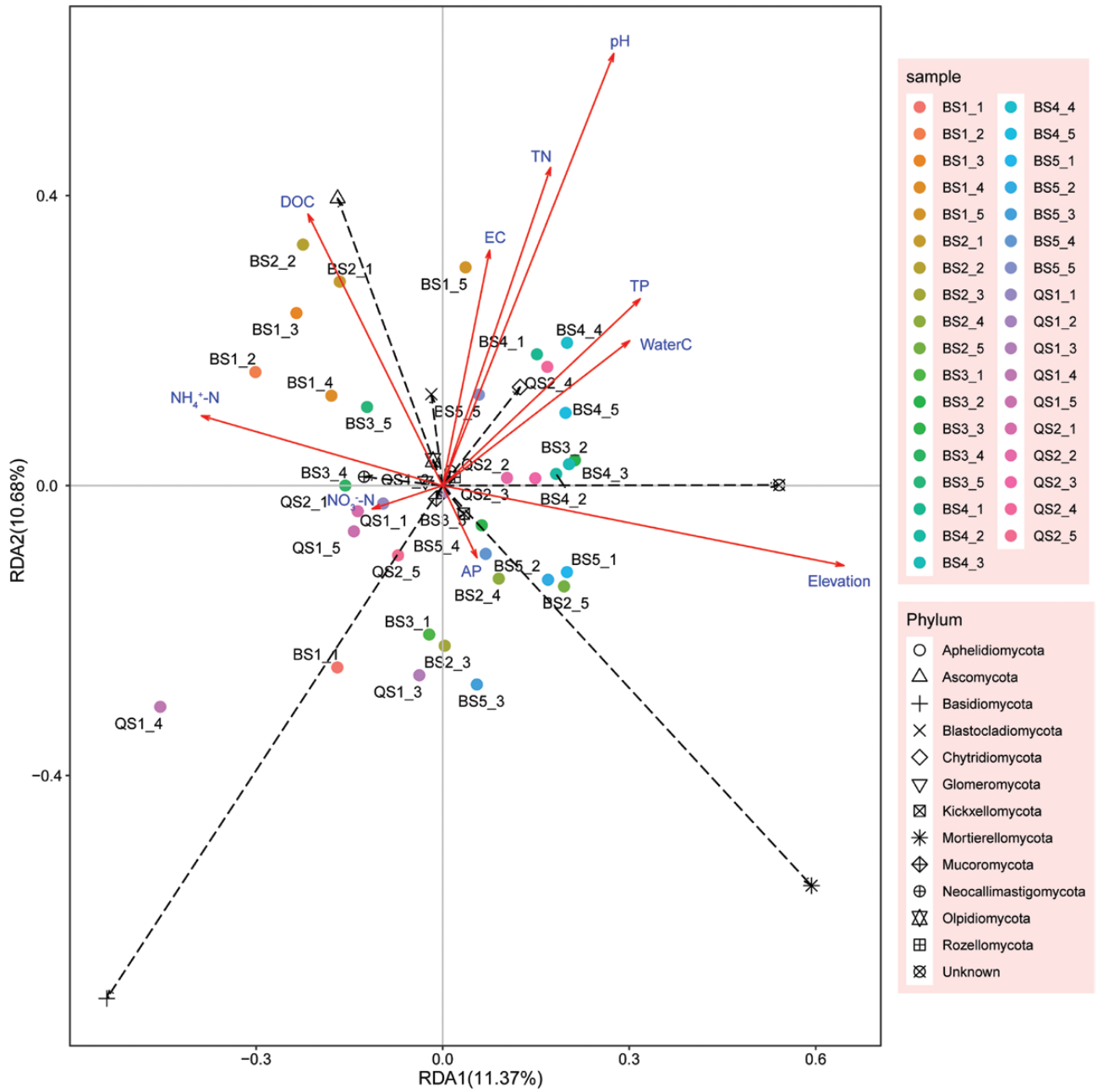

Figure 4: The tb-RDA analysis of rhizosphere fungal community. pH = soil pH.

positively related to the elevation (Supplementary Table S2). The OTU relative abundance of the saprotroph functional group was not significantly associated with any environmental factors (Supplementary Table S2). The OTU relative abundance of the symbiotroph functional group was negatively related to elevation (Supplementary Table S2).

The correlations between the fungal community of rhizosphere soil and root

The number of OTUs (R^2 = 0.12, P = 0.045, Fig. 6a), Simpson diversity index (R^2 = 0.12, P = 0.043, Fig. 6b) and Shannon–Wiener diversity index (R^2 = 0.22, P = 0.004, Fig. 6c) of the rhizosphere and root fungal communities were negatively correlated with each other. The OTU relative abundance of the rhizosphere and root fungal communities was not correlated with each other for most of the phyla except for Mucoromycota (P = 0.036, R = 0.244, Table 2) and Olpidiomycota (P = 0.043, R = 0.288, Table 2).

DISCUSSION

In this study, we investigated the changes in composition and diversity of rhizosphere and root fungal communities of *G. quadriradiata* along an elevational gradient in the

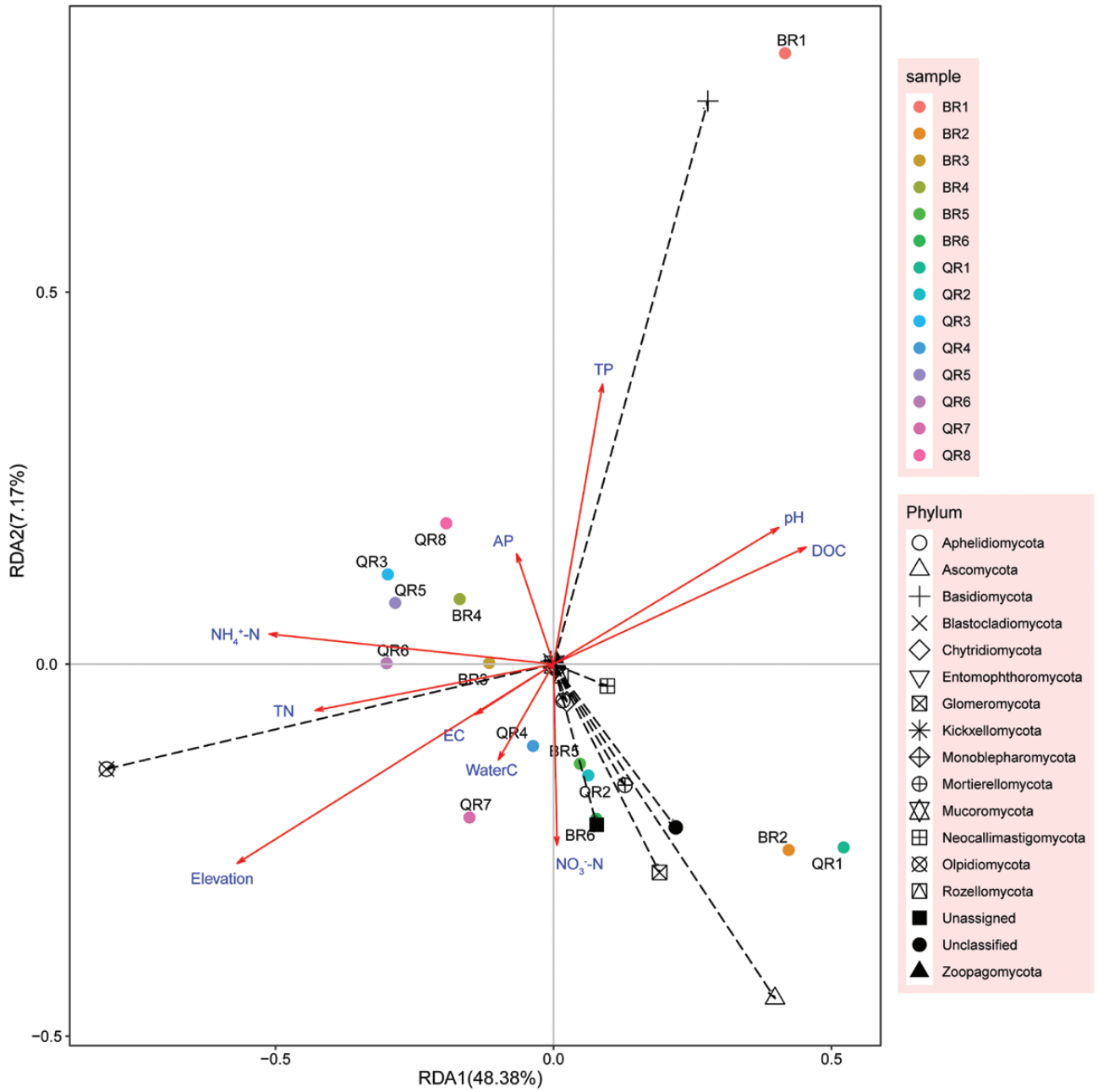

Figure 5: The tb-RDA analysis of root fungal community. pH = soil pH.

Qinling and Bashan Mountains. The fungal community diversity and OTU relative abundance in the rhizosphere increased while those in the root decreased with elevation (Fig. 2), suggesting that this invader is more selective about fungal interactions at higher elevations. Interestingly, the OTU relative abundance of pathogenic fungi in the rhizosphere decreased while that in the root increased with elevation (Fig. 3), suggesting that the fungi that maintain interactions at higher elevations are those that are generally considered detrimental to plant performance. The Beta diversity of fungal and AMF communities in rhizosphere and root was significantly different between high and low elevation (Supplementary Figs S1–S4). Our results indicate that the expansion of *G. quadriradiata* into high elevation is accompanied by shifts in the mycorrhizal community. The reduced interactions between symbiotic fungal species and *G. quadriradiata* may affect the invasion process of the invader at high elevations.

Different changing pattern of fungal community in the root and rhizosphere

In this study, root fungal community diversity significantly decreased with elevation while that

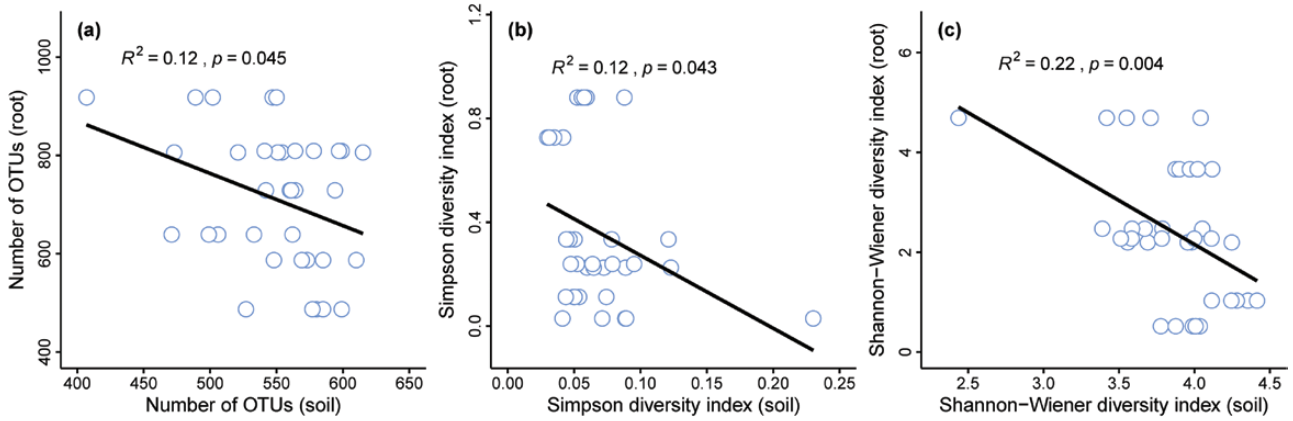

Figure 6: The linear correlations of fungal community diversity [(**a**) number of OTUs, (**b**) Simpson diversity, (**c**) Shannon-Wiener diversity] between rhizosphere and root of *Galinsoga quadriradiata*.

Table 2: These results of Pearson's correlation analysis for alpha diversity, number of OTUs, number of genera, the relative abundance of phyla and fungal functional groups between rhizosphere soil fungal community and root

Root *vs.* Soil	***t***	***df***	***P***	**Correlation**
Aphelidiomycota	1.286	33	0.208	0.218
Ascomycota	1.730	33	0.093	0.288
Basidiomycota	1.443	33	0.159	0.244
Blastocladiomycota	0.146	33	0.885	0.218
Chytridiomycota	−1.288	33	0.207	0.288
Glomeromycota	1.855	33	0.073	0.244
Mortierellomycota	−0.524	33	0.604	0.288
Mucoromycota	2.186	33	**0.036**	0.244
Neocallimastigomycota	1.414	33	0.167	0.218
Olpidiomycota	2.104	33	**0.043**	0.288
Rozellomycota	−1.031	33	0.310	0.244
OTU RA of pathotroph	−1.035	33	0.308	−0.177
OTU RA of saprotroph	−0.508	33	0.615	−0.088
OTU RA of symbiotroph	−1.629	33	0.113	−0.273

The correlation >0 means positive correlation, and <0 means negative correlation, the greater the absolute value, the stronger the correlation. Abbreviation: OTU RA = OUT relative abundance. Bold value indicates that it is statistically significant at $P = 0.05$ level.

of the rhizosphere samples significantly increased (Fig. 2). This demonstrates that the abundance of colonizing fungi decreases when *G. quadriradiata* expands to higher elevations. Furthermore, the OTU relative abundance of symbiotrophs did not change with elevation in the rhizosphere samples, but it significantly decreased with elevation in the root samples (Fig. 3). These results suggest that it is not fungal availability in the soil that changes across elevation, but rather the ability of the plant to create and maintain these associations decreases. This is presumably because of a trade-off between the invasive plant's growth and fungal symbiosis. For example, although many invasive plants closely interact with mycorrhizal fungi and benefit from the interactions, the association can be costly under certain conditions (Grove *et al.* 2017), resulting in situations where previously beneficial interactions become either less

beneficial or even harmful. Our results suggest that *G. quadriradiata* reduces the number of symbiotic fungal species it interacts with at higher elevations, which could potentially indicate that some previously beneficial mycorrhizal interactions become too costly to maintain. It indicates that the plants become more selective in which fungal species to interact with under stressful conditions. This latter hypothesis is supported by our quantification of Beta diversity (Supplementary Figs S1–S4), which shows that high-elevation sites tend to clump together thereby demonstrating high degrees of community similarity. The fact that the rhizosphere samples do not show this trend further suggests that it is the plant i.e. selecting for the species to interact with and that these results are not due to environmental filtering of fungal species.

The decreased beneficial mycorrhizal symbiosis may constrain the expansion of invasive plants over wide distances and altitudinal zones, as has been noted in previous research (Urcelay *et al.* 2017). Therefore, the diversity and relative abundance of the mycorrhizal fungal community may be a biological limiting factor affecting the spread of invasive plants to mountain ecosystems. Still, the inconsistent changing patterns of fungal diversity and the relative abundance of symbiotrophs in rhizosphere versus root samples indicate that the decreasing associations between *G. quadriradiata* and fungal symbionts (including AMF) at high elevations probably are not caused by the lack of symbiotic fungi in the soil (Figs 2 and 3), suggesting that it is the plant i.e. selecting against more diverse fungal communities. This is consistent with a similar result from a recent study which found that the AMF associating with invasive plant species were present across the whole elevation gradient (Clavel *et al.* 2021), suggesting that it is not mycorrhizal presence i.e. limiting interactions with invasive plant species. We speculate that these results could indicate that invasive plants are selecting for less diverse mycorrhizal communities at high elevations. Meanwhile, to some extent, the changing pattern of the interaction between invasive plant and fungal community may also be affected by abiotic factors.

It is important to note here some of the caveats and conditions of FUNGuild output as they pertain to the interpretation of our results. FUNGuild relies on third-party contributions from scientists and therefore is impacted by the amount of data made available to it, the taxonomic accuracy to which species are identified to, and the accuracy of natural history and autoecology used to classify them (Nguyen *et al.* 2016). Despite the continued need to improve these aspects of FUNGuild for use in ecological studies such as this, it remains the most useful tool we currently have at our disposal to do this type of analysis and remains widely used within the ecological literature allowing direct comparison to other studies within this field. Still, it is possible that deficiencies in these areas of concern could result in misclassification of fungal diversity, and thus misinterpretation of our results, within this study, and the conclusions we draw should be interpreted with that in mind.

Our results showed that the rhizosphere fungal community diversity of invasive *G. quadriradiata* increased with elevation (Fig. 2), suggesting that fungal abundance in the soil will likely not be a limiting factor in the elevational range expansion of this species in central China. A recent study also showed that the trend of rhizosphere fungal community diversity along elevation differed between different groups of fungi, with richness of root-inhabiting fungi interacting with a native tree decreasing at higher elevations (Park *et al.* 2021). The contrast between these results and our work suggest that one aspect of successful plant invasions at high altitude may include maintaining fungal diversity in the rhizosphere while being selective about which species colonize the roots.

This would be consistent with some studies that have suggested that invasive plants could alter rhizosphere fungal community and therefore strengthen their growth via plant–soil feedback (Batten *et al.* 2006; Caravaca *et al.* 2020; Inderjit and van der Putten 2010). Invasive plants have been shown to attract more soil fungi to establish a symbiotic network and subsequently promote their colonization in new environments (Batten *et al.* 2006; Fahey *et al.* 2020; Gomes *et al.* 2018; Inderjit and van der Putten 2010). For example, some invasive plants have positive impacts on the soil fungal communities when they spread into a new environment, thus leading to great plant performance and further invasion (Anthony *et al.* 2017; Wang *et al.* 2018). In addition, others have found that, because of higher root activity earlier in the growing season and different nutrition provision strategies by invasive plants, the soil AMF community differed dramatically between native and invasive species (Hawkes *et al.* 2006). Although the change in soil fungal community may be more conducive to the growth of invasive plants (Hawkes *et al.* 2006), invasive plants may still reduce fungal associations in response to the harsh abiotic

factors they face at high elevations and thus reduce the loss of carbohydrate to mycorrhizae.

Changes in diversity and abundance depend on mycorrhizal functional group

In the rhizosphere, OTU relative abundance of pathogens significantly decreased while abundance of symbiotrophs did not change significantly with elevation (Fig. 3). In contrast, root pathogen OTU relative abundance significantly increased while that of symbiotrophs significantly decreased with elevation (Fig. 3). Together, these results indicate that even though symbiotic fungal species remain available to *G. quadriradiata* at high elevations, the plant is limited by its ability to form associations with these species while simultaneously accumulating a greater proportion of pathogenic fungi. To this point, the OTU relative abundance of pathogens was generally higher than that of symbiotrophs both in the rhizosphere and root samples. This could reflect results from previous research which found that invasive plants accumulate many pathogenic fungi in the soil to inhibit the growth of competitors when they spread into new habitats (Klironomos 2002; van der Putten *et al.* 2007b). Most of these pathogenic fungi usually are generalist pathogens, and they are less harmful to invasive plants than native plants (Inderjit and Cahill 2015; Inderjit and van der Putten 2010). However, further study is still needed to confirm this point and demonstrate that performance of the invader is not strongly negatively affected. In addition, the OTU relative abundance of symbiotrophs showed a significantly negative trend with elevation in the root of *G. quadriradiata*, while showed a no significantly positive trend in the rhizosphere (Fig. 3). This result suggests that there may be antagonistic effects between pathogens and symbiotrophs which could affect the spread of invasive plant *G. quadriradiata*. Previous studies have documented antagonistic effects between soil fungal pathogen and symbiotic fungi (e.g. AMF) (Borowicz 2001; Liang *et al.* 2015). For example, the interactions between pathogen and symbiotroph weaken the growth of invasive *Robinia pseudoacacia* (Borowicz 2001; Callaway *et al.* 2011). Therefore, relatively greater proportions of pathogenic fungi at high elevations may inhibit the spread of invasive plants into mountain ranges.

The effect of environmental factors

Environmental factors can significantly affect the species composition and OTU relative abundance of the soil fungal community (Tedersoo *et al.* 2014). Previous studies suggest that elevation, nutrient content (e.g. phosphorus) (Bueno de Mesquita *et al.* 2018) and habitat filtering (Haug *et al.* 2019) could influence fungal community abundance (e.g. AMF), and thus could lead to fungal community turnover (Li *et al.* 2018). Our results show that the composition of the rhizosphere fungal community of *G. quadriradiata* is strongly related to elevation, soil pH, AP and TN (Fig. 4). The composition of the root fungal community was significantly correlated with DOC (Fig. 5). Together, this suggests that the changes in the fungal community can be explained by the changes of soil physical and chemical properties along the invader's expansion route (Saitta *et al.* 2018; van der Putten 2002; Wang *et al.* 2015) that result from a combination of both biotic and abiotic drivers.

Soil chemical and physical properties can modify the fungal community during plant invasions. For example, the enrichment of nutrient elements could lead to aggregations of fungal community in soils and roots (Phillips *et al.* 2019). Soil pH is a dominant driver of microbial community composition and is significantly related to fungal richness and alpha diversity (Wang *et al.* 2015). Environmental factors usually change sharply in mountain ranges: e.g. local microclimatic conditions (e.g. water) can vary largely even within a very short distance in the mountain range (Veach *et al.* 2018). Thus, soil fungal community composition and diversity usually show a strong shift along elevational gradients in response (Bahram *et al.* 2012; Haug *et al.* 2019; Urcelay *et al.* 2019). Our results indicate that the effects of environmental factors on the rhizosphere and root fungal communities are different, and the interaction between invasive plants and fungal community may change along its expansion route. However, the interaction between them is complicated, and remarkably affected by both biotic and abiotic factors. Due to the lack of evidence from reciprocal transplant experiment and controlled experiment, it is currently hard to separate the interactions between the plant invasion with soil biotic and abiotic properties. Further studies are still needed.

Correlations between the rhizosphere and root fungal community compositions

Generally speaking, the fungal community in roots is recruited from the surrounding soil fungal communities, and can be determined by host plant defense strategies, root structure and root exudation (Edwards *et al.* 2015; Lumibao *et al.* 2020). Previous

studies indicate that there are significant differences in fungal communities between rhizosphere and root communities, and both of them could affect the fungal composition of the other (Lopez-Angulo *et al.* 2020; Sietio *et al.* 2018). Our results indicate that the fungal community diversity and composition in the rhizosphere of *G. quadriradiata* were negatively correlated with those in the roots (Fig. 6). However, the relative abundance of some fungi species showed consistent trends in both rhizosphere and roots (Supplementary Tables S3 and S4). This suggests that the fungal community in the rhizosphere and roots may interact with each other during the expansion of *G. quadriradiata* into high elevation. Put another way, even though fungal diversity changed in opposite directions between the two sources, the fact that certain species showed consistent trends regardless of source suggests that there is still strong interaction between the two. This interaction between rhizosphere soil and roots is an interesting mechanism which is of great significance for the colonization and diffusion of invasive plants. The differences in fungal communities between roots and rhizosphere may be due to the selection of invasive plants, and they prefer to interact with these species which benefit them. There is a trade-off between carbohydrate loss and growth of invasive plants. For example, invasive plant *Eupatorium catarium* and *Bidens pilosa* interact more strongly with AMF under poor nutrient status than under rich nutrient status (Chen *et al.* 2020). Then, invasive plants may select different fungal species to interact with under different conditions. Still, further research is needed to reveal the specific mechanism and its effect on plants and the fungal community and their interrelationship.

CONCLUSIONS

We investigated the changes in rhizosphere and root fungal community of invasive plant *G. quadriradiata* along its expansion route in mountain ranges. Consistent with our research hypothesis, the species composition and relative abundance of rhizosphere and root fungal community changed significantly along elevational gradients. Our results show that the fungal diversity in the rhizosphere increased significantly during the spread of *G. quadriradiata* into high elevation, indicating that the invasive plant may not suffer from limited fungal symbionts in the soil when it colonizes into high altitudes. However, at the same time, the species diversity and abundance of root fungi decreased with elevation, suggesting the interactions between the invader and fungi could be compromised under the same conditions. Moreover, the abundance of fungal symbiotrophs in the roots also decreased with elevation while that in the rhizosphere did not change with the elevation, suggesting that the dependence of the invasive plant on the fungal symbionts decreases with the elevation. The OTU relative abundance of rhizosphere pathotrophs decreased while that in the roots increased with the elevation, indicating that the invader tends to accumulate more pathogenic fungi in the roots when it expands along elevation. In sum, our results highlight that the interactions between invasive plants and fungal community substantially changed when the invader expanded into high-elevational areas and suggesting that belowground interactions will be key in our understanding of how invasive plants derive success in stressful, high-elevation environments.

Supplementary Material

Supplementary material is available at *Journal of Plant Ecology* online.

Table S1: Pearson's correlation coefficients between environmental factors and rhizosphere fungal community at the phylum level.

Table S2: Pearson's correlation coefficients between environmental factors and root fungal community at the phylum level.

Table S3: Pearson's correlation coefficients between elevation and rhizosphere fungal community at the genus level.

Table S4: Pearson's correlation coefficients between elevation and root fungal community at the genus level.

Figure S1: The Beta diversity of the fungal community in rhizosphere of *Galinsoga quadriradiata* at different elevations.

Figure S2: The Beta diversity of AMF community in rhizosphere of *Galinsoga quadriradiata* at different elevations.

Figure S3: The Beta diversity of the fungal community in the root of *Galinsoga quadriradiata* at different elevations.

Figure S4: The Beta diversity of AMF community in the root of *Galinsoga quadriradiata* at different elevations.

Figure S5: The fungal community composition and OTU relative abundance in rhizosphere of *Galinsoga quadriradiata* at the phylum level.

Figure S6: The fungal community composition and OTU relative abundance in rhizosphere of *Galinsoga quadriradiata* at the family level.

Figure S7: The fungal community composition and OTU relative abundance in the root of *Galinsoga quadriradiata* at the phylum level.
Figure S8: The fungal community composition and OTU relative abundance in the root of *Galinsoga quadriradiata* at the family level.
Figure S9: The linear regression between elevation and OTU relative abundance (OTU RA) of AMF in rhizosphere (**a**) and root (**b**) of *Galinsoga quadriradiata*.

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Conflict of interest statement. The authors declare that they have no conflict of interest.

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