



ORIGINAL ARTICLE OPEN ACCESS

Resource Allocation Trade-Offs and Rewired Mycorrhizal Networks Underlie the Adaptation of *Paphiopedilum purpuratum* to Ex Situ Conservation

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Received: 25 October 2025 | **Revised:** 6 January 2026 | **Accepted:** 3 March 2026

Keywords: carbon-nitrogen metabolism | ex situ conservation | fungal community | mycorrhizal fungi | *Paphiopedilum purpuratum* | photosynthetic adaptation | physiological response

ABSTRACT

This study reveals how the rare orchid *Paphiopedilum purpuratum* adapts to ex situ conservation. Although seed production was significantly increased, the plants incurred physiological costs: reduced photosynthesis and higher oxidative stress. Importantly, host physiology—particularly nitrogen metabolism—directly influenced root fungal communities. This drove two distinct adaptive strategies in the fungal community. Mycorrhizal fungi maintained stable communities by reorganizing their interaction networks into resilient multi-cluster structures. In contrast, non-mycorrhizal fungi exhibited high species turnover, shifting toward beneficial taxa that potentially suppress pathogens. These findings highlight that successful ex situ conservation must address both the plant physiological trade-offs and its restructured root microbiomes. We propose that the orchid's resilience relies on a multi-level microbial adaptation strategy, combining a reorganized yet stable mycorrhizal core with a dynamic, beneficial non-mycorrhizal periphery. Our work offers a new framework for protecting rare orchids—one that prioritizes sustaining these complementary fungal partnerships to ensure long-term survival.

1 | Introduction

In an era of accelerating biodiversity loss, ex situ conservation serves as a critical safeguard against species extinction. Yet traditional metrics of its success—notably survival and fecundity rates—suffer from fundamental limitations [1, 2]. These conventional metrics, however, focus primarily on short-term survival and reproduction, failing to capture the underlying functional integrity essential for long-term population viability and evolutionary potential [3]. For plants that depend heavily on complex biotic interactions—such as orchids—ex situ conditions often alleviate one set of constraints while introducing novel biotic or abiotic stressors. This can allow plants to achieve

seemingly high reproductive success, yet at a hidden cost: physiological dysregulation and degradation of mutualistic networks [4, 5]. Given these challenges, conventional assessment tools are increasingly inadequate for meeting the scientific demands of contemporary ex situ conservation. Consequently, conservation biology urgently requires a new evaluation paradigm—one that moves beyond superficial metrics to diagnose the systemic costs of adaptation [6, 7].

Orchids exemplify a life history of “dual dependence”: seed germination relies strictly on specific mycorrhizal fungi for carbon and nutrient supply, whereas sexual reproduction depends on highly specialized pollinators [8, 9]. This intricate interaction

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Highlights

- Under ex situ conservation, the endangered orchid *Paphiopedilum purpuratum* achieves reproductive success through stabilized mycorrhizal associations and optimized carbon-nitrogen metabolism. However, insufficient photosynthetic adaptation remains a major constraint on its long-term survival.

network predisposes orchids to a “flourishing yet maladaptive” dilemma under ex situ conditions. Studies show that many translocated orchids exhibit symptoms such as flowering failure, suppressed vegetative growth, reduced stress tolerance, and poor seedling establishment—outcomes often traced to disruptions or dysfunctions in their interaction networks [10–12]. The endangered slipper orchid *Paphiopedilum purpuratum* illustrates this challenge: its wild populations, critically vulnerable to habitat degradation, make ex situ conservation an essential intervention [13–17]. Current conservation practice faces two critical mechanistic gaps. First, its specialized pollination system remains poorly understood, resulting in extremely low natural pollination efficiency [18, 19]. Second, under ex situ conditions, the functional status, assembly rules, and physiological coupling of its symbiotic fungal communities with the host plant remain largely unknown [20, 21]. This dual knowledge gap confines conservation management to a “black-box” approach—reliant on empirical practices without mechanistic insight—hindering the transition from merely keeping plants alive to ensuring their long-term health and adaptability.

To move beyond this black-box approach and diagnose systemic adaptation costs, a theoretical framework explicitly linking aboveground reproductive output with belowground function is required [22]. We propose the stress-induced resource allocation trade-off model as such a framework [23]. This model posits that under ex situ stress, plants face a critical allocation dilemma: limited internal resources (e.g., carbon, nitrogen) must be partitioned between immediate reproductive effort and long-term investments in physiological homeostasis and mutualistic symbioses [24, 25]. Consequently, increased investment in reproduction may occur at the expense of physiological resilience and symbiotic network integrity. Thus, to comprehensively diagnose bottlenecks in ex situ adaptation, it is essential to simultaneously evaluate reproductive success, host physiological state, and the structure and function of symbiotic communities [26].

This juxtaposition suggests a core hypothesis: ex situ stress triggers a resource allocation trade-off, diverting limited resources from symbiotic and long-term physiological investments toward securing immediate reproduction. To map the baseline patterns of this potential trade-off, we conducted a synchronous multidimensional comparison of wild and ex situ populations of *P. purpuratum*, quantifying reproductive performance, root fungal community structure, and key host physiological indicators. Our study aimed to (1) quantify the impact of ex situ cultivation on reproductive output and (2) characterize the coupled response patterns between host physiology and fungal communities.

This work provides the first comprehensive phenotypic dataset on *P. purpuratum*'s ex situ adaptation. Although it does not

directly test causal mechanisms, the dataset establishes a critical empirical baseline for evaluating the resource trade-off hypothesis and guiding the design of conservation strategies aimed at sustaining systemic plant health rather than mere survival.

2 | Materials and Methods

2.1 | Plant Materials and Habitat Description

The materials used in this study were collected from Zijin County (Heyuan City, Guangdong Province, China). This region exhibits a southern subtropical monsoon climate with medium-low mountain and hill terrain (maximum altitude 1337 m) and native sandy yellow-red soil. Local climate records indicate an annual mean temperature of 22.1°C, annual precipitation of 3428.9 mm, relative humidity > 80%, annual sunshine duration of 1743.4 h, and a frost-free period of 350 days, collectively creating a shaded understory habitat with high humidity and low light intensity. The original habitat of *P. purpuratum* is illustrated in Figure 1, Panels A-1 and A-2.

Ex situ conservation of *P. purpuratum* was established in an introduction greenhouse at the South China National Botanical Garden. Plants were maintained under simulated in situ habitat conditions with controlled temperature, humidity, and light intensity. The growth medium comprised a 1:1 (v/v) mixture of humus soil and pine bark. Inside the greenhouse, the mean annual temperature was maintained at 27°C and relative humidity was regulated between 65% and 70%. Irrigation was adjusted seasonally: during spring, summer, and autumn, plants were sprinkler-irrigated for 5 min every 2 days, alongside a water-curtain ventilation system to sustain optimal air humidity; in winter, irrigation frequency was reduced to once every 3–4 days. Panels B-1 and B-2 of Figure 1 depict the ex situ conservation greenhouse established for this study.

2.2 | Sample Collection

P. purpuratum was sampled from its native habitat and an ex situ conservation nursery where plants had been acclimatized for at least 3 years old. All sampling in both the native habitat and the conservation greenhouse was conducted during the peak physiological activity period (April–June 2024). Healthy individuals were randomly selected. From each plant, 2–4 mature leaves and 3–5 cm root segments were collected, transported on ice to the laboratory, and all processing was completed within 24 h. Following surface sterilization (2 min in 5% NaClO, 30 s in 75% ethanol, three rinses with sterile water, and drying on sterile filter paper), the roots were observed. Roots displaying mycelial clusters in segments and cross-sections (Figure 1C-2) were classified as mycorrhizal, whereas those without (Figure 1C-1) were classified as non-mycorrhizal for subsequent analysis. Subsamples were allocated for different analyses: leaves for physiological assays; both leaves and roots for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope determination; and root segments for ITS2 amplicon sequencing to characterize the endophytic fungal community.

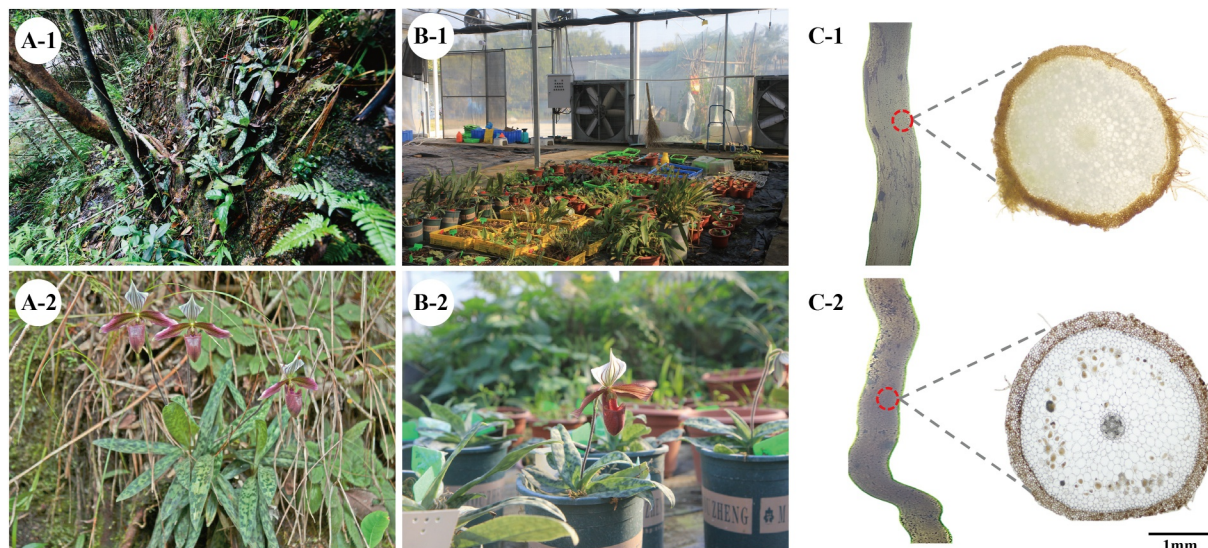


FIGURE 1 | *P. purpuratum* under in situ and ex situ conservation. (A-1) Original habitat of the species; (A-2) Plant individuals under in situ conservation; (B-1) Environment of the ex situ conservation greenhouse; (B-2) Plants transplanted in flower pots under ex situ conditions; (C-1) Non-mycorrhizal roots, showing a translucent surface and absence of hyphae in cross-section; (C-2) Mycorrhizal roots, exhibiting a darker surface color and presence of hyphae in cross-section.

A paired sampling design was adopted, with root and leaf tissues collected from the same individual to enable matched cross-dimensional analyses of physiological traits, isotopic signatures, and microbiome composition. Plant selection followed a randomized approach within accessible wild and ex-situ populations; individuals within each group were sampled from distinct ramets to minimize shared genetic background. To control for within-plant variability, tissues were sampled from physiologically mature, sun-exposed positions. Given the critically endangered status of *P. purpuratum* and the limited number of healthy flowering individuals, the sample size was restricted to $n = 3$ biological replicates per condition (site $\times$ tissue). This sample size is suitable for the exploratory objective of identifying cross-scale association patterns; the analysis focuses on individual-level data and employs statistical methods robust to ecological heterogeneity, such as β -diversity and ordination techniques.

2.3 | Determination of Breeding Biological Characteristics

Flowers from ten flowering individuals were selected for morphological observation. Key floral traits, such as flower color, were recorded and photographed. For ultrastructural analysis, pollen grains and stigmas were fixed in glutaraldehyde-paraformaldehyde, rinsed in phosphate buffer, dehydrated through a graded ethanol series, critical-point dried, and sputter-coated with platinum prior to scanning electron microscopy (SEM). Pollen viability and stigma receptivity were assessed at 1, 3, 5, 7, 9, and 11 days post-anthesis using acetocarmine staining and the benzidine-hydrogen peroxide method, respectively. Seed viability was examined based on the morphology of seeds collected from mature capsules and determined using the bromothymol blue (BTB) staining method (four replicates of 100 seeds each). To evaluate pollination efficiency, 93 flower buds in the native habitat were assigned to three treatments: 56 flowers

for artificial cross-pollination, 26 for artificial self-pollination, and 11 untreated controls. In the ex situ greenhouse, 67 flowers were used: 36 for cross-pollination and 31 for self-pollination. The seed set rate was calculated as the proportion of mature capsules obtained in each treatment group.

2.4 | Measurement of Photosynthetic and Physiological Parameters

Photosynthetic parameters were measured using an LI-6400/XT portable photosynthesis system on healthy, fully expanded leaves from both wild and ex situ greenhouse conditions. Recorded parameters included net photosynthetic rate (A), intercellular CO₂ concentration (C_i), transpiration rate (E), stomatal conductance (g_{sw}), leaf temperature (T_{leaf}), leaf-to-air vapor pressure deficit (VPD_{leaf}), chamber relative humidity (RH_{cham}), and absorbed photosynthetically active radiation (R_{abs}). Each leaf was measured 10 times, with the mean value used for analysis. For biochemical assays, uniform leaf samples were homogenized in ice-cold extraction buffer, centrifuged, and the supernatant was collected as the crude enzyme extract. The following indices were measured in triplicate: glutathione reductase (GR; microassay), catalase (CAT; JL-T0900 kit), soluble sugar (SS; anthrone colorimetry), total antioxidant capacity (T-AOC; FRAP method), superoxide dismutase (SOD; WST-8 method), soluble protein (SP; Coomassie brilliant blue G-250 method), thiobarbituric acid (TBA; enzymatic colorimetry), malondialdehyde (MDA; TBA method), peroxidase (POD; guaiacol colorimetry), and chlorophyll (Chl; ethanol extraction spectrophotometry).

2.5 | Stable Isotope Analysis of Carbon and Nitrogen

Root and leaf samples were collected from native and ex situ habitats. A full factorial experimental design with three

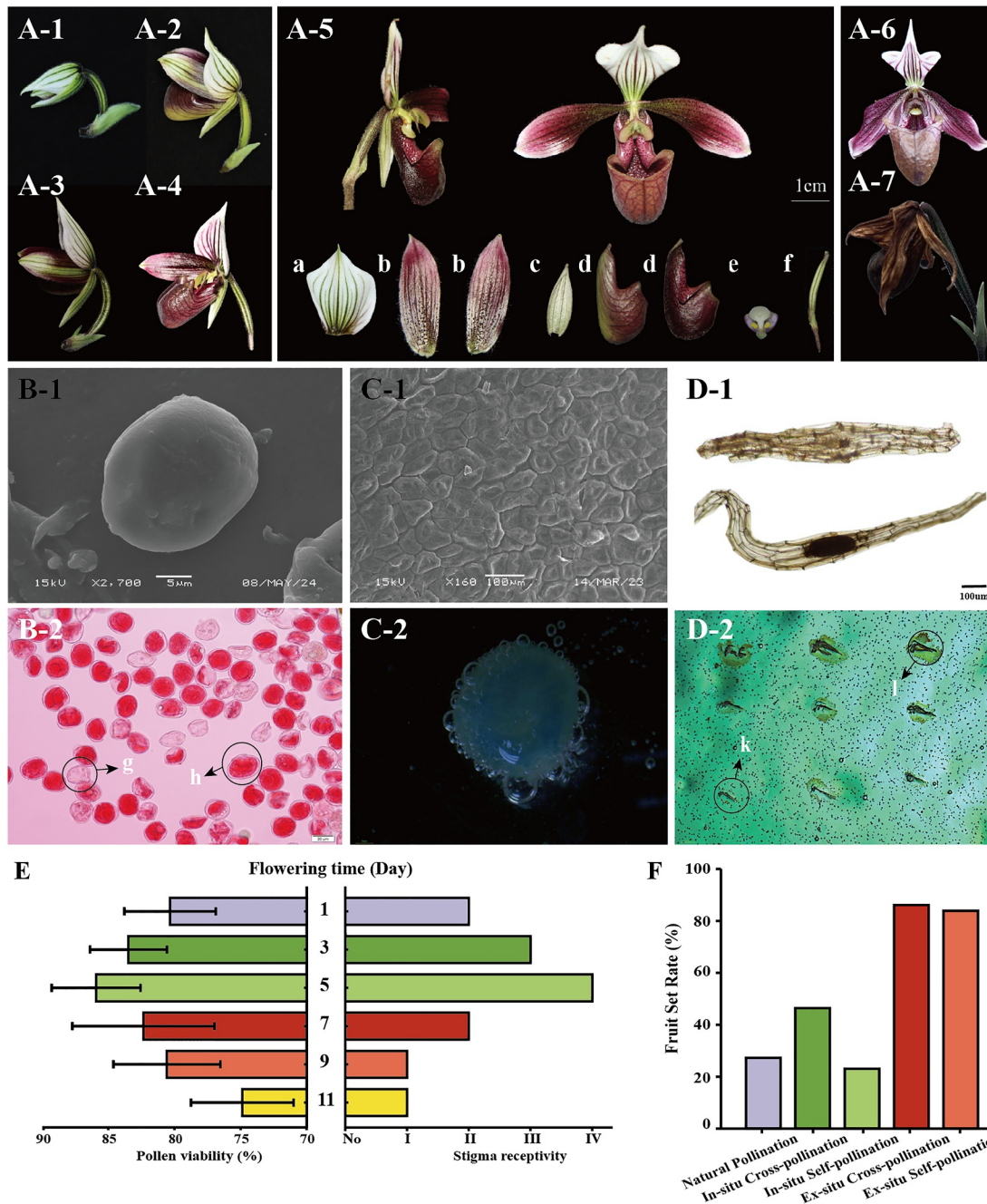


FIGURE 2 | Reproductive traits of *P. purpuratum*. (A-1–A-4) Flower development from bud to anthesis (Bud Stage 1, Bud Stage 2, Early Flowering Stage 1, Early Flowering Stage 2). (A-5) Fully opened flower with floral structures labeled: (A) dorsal sepal, (B) petal, (C) synsepal, (D) labellum, (E) column-related structure, (F) ovary. (A-6) Late flowering stage. (A-7) Senescent stage. (B-1) Scanning electron micrograph of pollen grains. (B-2) Acetocarmine-stained pollen at 5 days post-anthesis; (g) aborted pollen; (h) viable pollen. (C-1) SEM image of the stigma surface. (C-2) Stigma receptivity tested using the benzidine–hydrogen peroxide method; bubble formation indicates receptivity (5 days post-anthesis). (D-1) Seed morphology: embryonated versus non-embryonated seeds. (D-2) Seed viability assessed using BTB staining: (k) non-viable seed; (i) viable seed. (E) Dynamics of pollen viability and stigma receptivity (+: weak; ++: moderate; +++: strong; ++++: very strong; -: no data). (F) Seed-set rates under different pollination treatments between in situ and ex situ conservation.

replicates per tissue-habitat combination yielded 12 sample groups. Samples were vacuum-dried at 60°C, ground, and sieved through a 200-mesh sieve. Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios were determined using an elemental analyzer coupled with an isotope-ratio mass spectrometer (EA-IRMS). Powdered samples were combusted at 1020°C, and isotope ratios were calibrated against the Vienna Pee Dee Belemnite

VPDB standard (for $\delta^{13}\text{C}$) and atmospheric N_2 (for $\delta^{15}\text{N}$). Values were calculated using standard equations:

$$\delta^{13}\text{C} = (R_{\text{standard}} / R_{\text{sample}} - 1) \times 10^3 (\text{‰}, \text{VPDB})$$

$$\delta^{15}\text{N} = (R_{\text{standard}} / R_{\text{sample}} - 1) \times 10^3 (\text{‰}, \text{Air-N}_2)$$

where R represents the ratio of $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. Isotopic values were compared with published data from ecologically similar orchid species [27, 28]. All analytical procedures complied with the ISO/IEC 17025 laboratory quality management standard, with measurement uncertainties maintained below 0.15‰ for $\delta^{13}\text{C}$ and 0.3‰ for $\delta^{15}\text{N}$.

2.6 | High-Throughput Sequencing of Root Endophytic Fungi

Root samples (including mycorrhizal and non-mycorrhizal segments) were collected from native and ex situ habitats (three replicates per condition, total 12 samples). Genomic DNA was extracted using the CTAB method and purified with an Omega Bio-Tek E.Z.N.A. kit. The ITS2 region was amplified (Phusion High-Fidelity PCR Master Mix; 0.2 μM primers, 10 ng DNA) under the following PCR protocol: 98°C for 1 min; 30 cycles of 98°C/10 s, 55°C/30 s, and 72°C/30 s; final extension 72°C/5 min. Amplicons were verified on 1.5% agarose gels, purified with AMPure XP beads, and quantified with a Qubit 4.0 fluorometer. Libraries were constructed using the NEB Next Ultra II FS DNA Library Prep Kit and sequenced on an Illumina NovaSeq 6000 platform (paired-end 250 bp, PE250) after quality assessment on an Agilent 2100 Bioanalyzer. Raw reads were filtered with Trimmomatic v0.39 ($Q < 20$). Operational taxonomic units OTUs were clustered at 97% similarity via USEARCH v11 (Uparse), and taxonomic assignment was performed by BLASTn against UNITE v8.2 and NCBI nt databases ($e\text{-value} \leq 1 \times 10^{-5}$).

2.7 | Statistical Analysis

Data were analyzed using GraphPad Prism 10.1 and R (v4.3.0). Group comparisons of physiological and isotopic data were performed using two-way ANOVA. Microbial community diversity, structure (PCoA based on Bray-Curtis distance), and differential abundance were analyzed using standard packages (vegan, DESeq2). Community assembly processes were inferred using βNTI and RC-Bray metrics. Associations between host traits and fungal communities were examined using redundancy analysis (RDA, with variable screening by VIF), random forest, Mantel tests, and within-group correlations. Fungal co-occurrence networks were constructed and their topological properties were analyzed (igraph); network robustness was assessed using node/edge removal simulations.

3 | Results and Analysis

3.1 | Reproductive Traits of *P. purpuratum*

The flowers of *P. purpuratum* are purple-maroon with distinct dark purple venation and spotting, featuring a helmet-shaped labellum of the same color (Figure 2A). Under ex situ greenhouse conditions, floral buds began to form in September and senesced by November. Pollen grains exhibited smooth surfaces and retained detectable viability starting from the first day post-anthesis, with peak viability observed on day 5 (Figure 2B–2E).

The stigma exhibited a textured, wrinkled surface and reached its maximum receptivity on day 5 post-anthesis (Figure 2C–2E). Seeds were classified into embryonated (with embryos) and non-embryonated types (Figure 2D1). After three months of dry storage, seed viability remained high at $88 \pm 0.82\%$ (Mean $\pm$ SD; Figure 2D–2). The species exhibited both self-pollination and cross-pollination capacities, with ex situ conservation leading to a significant 52% increase in seed-set rate compared with natural (in situ) conditions (Figure 2F).

3.2 | Species Composition and Diversity of Root-Inhabiting Fungi

Following ex situ transfer, non-mycorrhizal fungal communities underwent pronounced restructuring, characterized by significant shifts in the relative abundances of Mortierellomycota and Rozellomycota, and significant alterations in dominant OTU composition (Figure 3A,B). In contrast, mycorrhizal fungal communities maintained compositional and structural stability, exhibiting no notable changes in dominant OTUs between in situ and ex situ conservation. Differential abundance analysis further highlighted this divergence, revealing substantial shifts in enriched OTUs within non-mycorrhizal communities, whereas mycorrhizal communities displayed only minor fluctuations (Figure 3C). The species richness of non-mycorrhizal fungi increased significantly post-transplantation, whereas the Shannon index remained stable—indicating community reorganization without loss of overall diversity. Mycorrhizal communities, however, showed no significant variation in either observed species or the Shannon index, reflecting preserved diversity and structural integrity (Figure 3D). Principal Coordinates Analysis (PCoA) corroborated these patterns: non-mycorrhizal communities showed clear separation along the PCo1 axis, consistent with habitat-driven restructuring, whereas mycorrhizal communities exhibited partial overlap along the same axis and a markedly lower separation distance—highlighting their greater resilience to environmental change under ex situ conditions (Figure 3E).

3.3 | Assembly Mechanisms of Fungal Communities Under Ex Situ Conservation

Analysis of species abundance distributions, βNTI —RC-Bray relationships, and ecological process contributions revealed key assembly mechanisms. All groups exhibited log-normal abundance patterns, supporting a key role of ecological drift (Figure 4A). After ex situ transfer, high-abundance species increased by 3.62% in non-mycorrhizal communities but decreased by only 1.52% in mycorrhizal communities, suggesting mycorrhizal networks buffer abundance shifts. Both community types exhibited improved goodness-of-fit of abundance distributions and larger metacommunity sizes under ex situ conditions, indicating reduced deterministic control. Non-mycorrhizal communities exhibited marginally higher βNTI values under ex situ conditions, implying stronger deterministic filtering. In contrast, mycorrhizal communities maintained βNTI values within $|\pm 2|$ and low RC-Bray dispersion, underscoring stochastic dominance and structural stability.

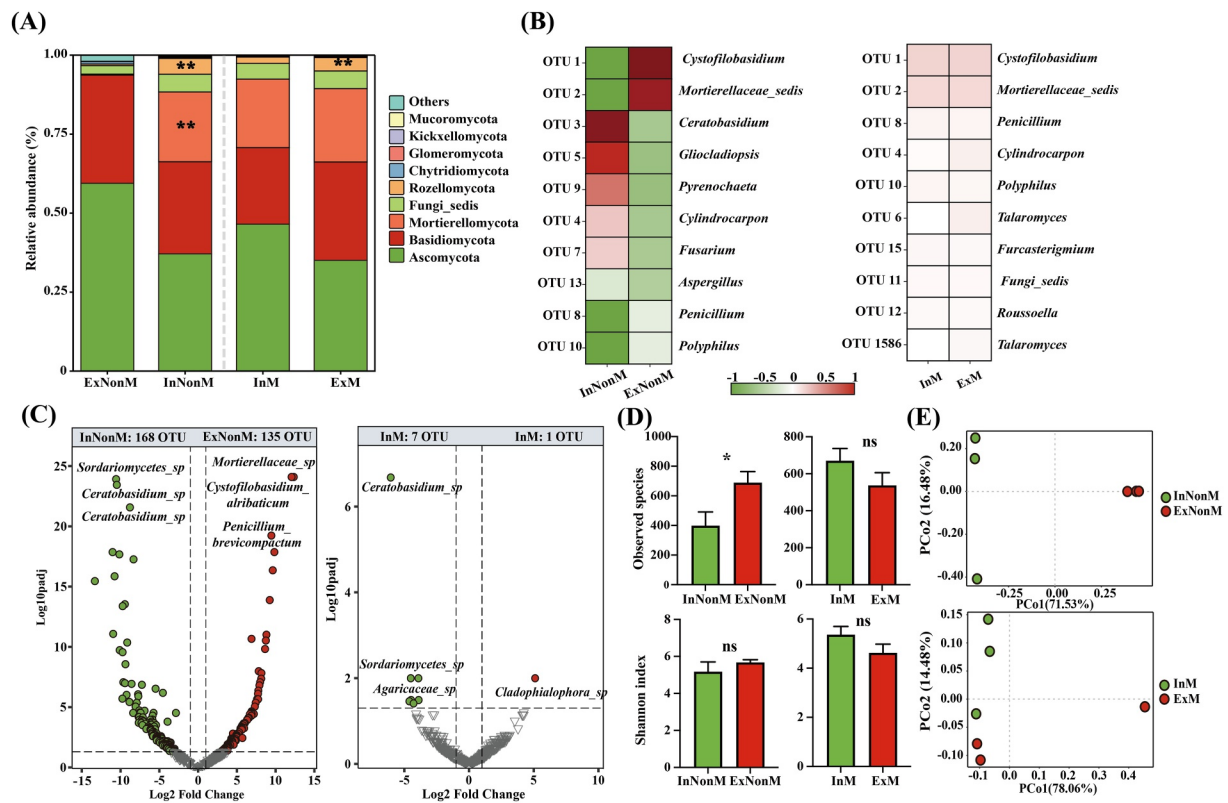


FIGURE 3 | Effects of ex situ conservation on mycorrhizal and non-mycorrhizal fungal communities in *P. purpuratum*. (A) Relative abundance of fungal taxa across groups (ExNonM: ex situ non-mycorrhizal; InNonM: in situ non-mycorrhizal; InM: in situ mycorrhizal; ExM: ex situ mycorrhizal). ** $p < 0.01$ indicates extremely significant differences. (B) Heatmap of dominant OTUs. (C) Volcano plots showing differentially enriched OTUs in non-mycorrhizal (left) and mycorrhizal (right) communities between in situ and ex situ conditions. (D) α -Diversity indices (observed species and Shannon index). * $p < 0.05$ indicates significant differences; ns: not significant ($p > 0.05$). (E) Principal Coordinates Analysis (PCoA) based on Bray–Curtis distances, showing structural differentiation among fungal communities.

(Figure 4B). Post-transfer, dispersal limitation was more prominent in non-mycorrhizal community assembly, whereas homogenizing dispersal more strongly shaped mycorrhizal communities (Figure 4C).

3.4 | Differential Network Responses to Conservation Modes

Analysis of the top 100 OTUs showed a slight decrease in negative correlations in non-mycorrhizal networks but a marked increase in mycorrhizal networks, indicating heightened resource competition in the latter (Figure 5A). Non-mycorrhizal networks retained more taxa with higher stability, whereas mycorrhizal networks contained fewer taxa and higher structural variability (Figure 5B). Topologically, non-mycorrhizal networks maintained a core–periphery structure; mycorrhizal networks shifted from single-core to multi-small-cluster forms (Figure 5C). No significant global topological differences emerged between sub-networks following ex situ conservation (Figure 5D). Robustness analysis revealed greater core-node resistance but higher edge vulnerability in non-mycorrhizal networks, whereas mycorrhizal networks exhibited stronger edge tolerance, and in situ mycorrhizal networks were highly sensitive to core-node deletion (Figure 5E). Post-conservation structural simplification

occurred: non-mycorrhizal networks retained aggregated modules with reduced complexity, whereas mycorrhizal networks developed sparser connectivity (Figure 5F).

3.5 | Host Physiological and Metabolic Adaptation Under Ex Situ Conservation

P. purpuratum showed intermediate $\delta^{13}\text{C}/\delta^{15}\text{N}$ signatures (Figure S1A1–2). Post-conservation increases in leaf/root $\delta^{13}\text{C}$ (Figure S1A–3,A–5) and decreases $\delta^{15}\text{N}$ (Figure S1A–7,A–9) indicated metabolic shifts, supported by elevated root nitrogen (Figure S1A–10). Physiologically, reduced light intensity decreased photosynthesis (Figure S1B–1,B–8). A drier, cooler microenvironment increased water loss (Figure S1B–2~4,S1B–6), whereas stomatal adjustments helped sustain CO_2 uptake under stress (Figure S1B–5,B–7). Stress tolerance was characterized by low oxidative damage (Figure S1C–1~2), elevated antioxidants (Figure S1C–2,S1C–7~8), and soluble sugar accumulation (Figure S1C–10). Photosynthetic function persisted despite lower chlorophyll content, as evidenced by a stable Chl a/b ratio (Figure S1C–4), whereas soluble proteins showed no significant change (Figure S1C–9). These physiological and metabolic responses reflect coordinated adaptation to ex situ conservation conditions.

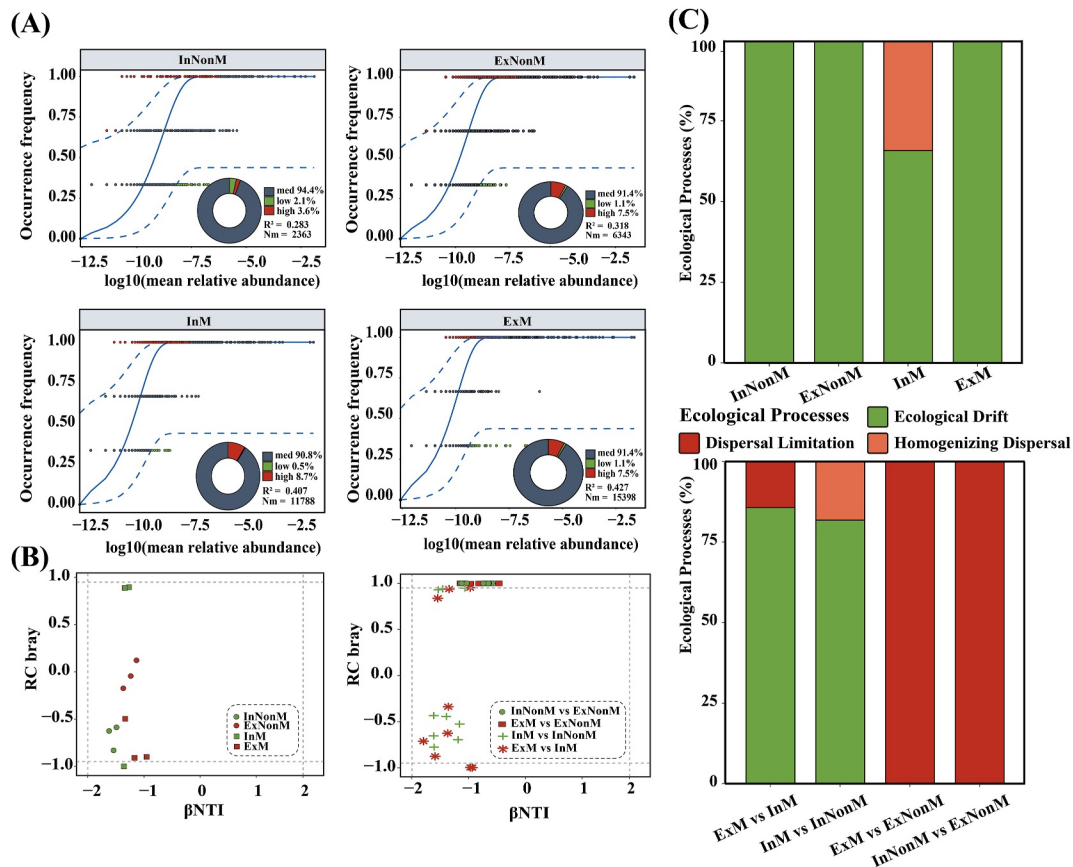


FIGURE 4 | Assembly processes of root-associated fungal communities in *P. purpuratum*. (A) Species abundance distributions (SADs) across treatments, showing proportions of low-, medium-, and high-abundance taxa, with fitted parameters R^2 (goodness of fit) and N_m (number of taxa). (B) Relationship between β NTI and RC-Bray across different comparison groups. (C) Relative contributions of ecological processes (dispersal limitation, homogenizing dispersal, ecological drift) to fungal community assembly under different treatments (upper panel) and comparison groups (lower panel).

3.6 | Reconfiguration of Host Phenotype and Fungal Community Associations Driven by Conservation Regimes

Integrated analyses (RDA, random forest, Mantel tests) demonstrated that conservation regimes reshape the host-fungus system. Although preceding analyses distinguished fungal communities by their symbiotic lifestyle (mycorrhizal vs. non-mycorrhizal), here we assessed how the conservation environment (in situ vs. ex situ) as a whole reconfigures the association network between host phenotype and the entire root fungal assemblage. RDA and random forest identified distinct host profiles: in situ was linked to enhanced osmotic adjustment (SS), antioxidant capacity (SOD), and nitrogen signature ($\delta^{15}\text{N}$); ex situ was associated with higher water-use efficiency ($\delta^{13}\text{C}$) and lipid peroxidation (MDA) (Figure 6A–B). Key fungal taxa significantly covaried with these traits. Mantel tests confirmed that host phenotype drives community structure ($R = 0.71$, $p = 0.06$), with leaf $\delta^{15}\text{N}$ as the strongest driver ($p < 0.01$), implicating nitrogen metabolism in fungal divergence (Figure 6C). Grouped correlation analysis revealed reversed correlations: total chlorophyll content (TCC) and Simpson diversity correlated positively in situ but negatively ex situ (Figure 6D). This directional shift indicates that ex situ conservation not only modifies host physiology and fungal community structure but also reprograms their interaction ecology.

4 | Discussion

4.1 | Reproductive Advantages Coexist With Inherent Constraints

Ex situ conservation has conferred significant reproductive benefits on *P. purpuratum*. Within the stable greenhouse microenvironment, its seed-set rate increased to 80%, substantially exceeding the 3% observed under natural pollination and 30% with artificial supplementary pollination in the wild [29–31]. This confirms that protected cultivation effectively alleviates reproductive bottlenecks caused by pollinator limitation and environmental stochasticity. However, core constraints persist despite this reproductive success. First, reproduction involves a “dual dependency”: successful fertilization depends on unidentified effective pollinators, while seed germination requires specific mycorrhizal fungi [32, 33]. Second, the plant exhibits clear trade-offs at both physiological and symbiotic levels: reduced net photosynthetic rate and chlorophyll content limit carbon gain [34–36]; meanwhile, the mycorrhizal fungal network has become more variable, exhibiting a topological shift toward multiple small clusters. Although the non-mycorrhizal fungal community shifted toward beneficial taxa, its assembly process remains unstable [37]. This duality—high reproductive success coupled with multilevel adaptive challenges—clearly indicates that conservation strategies must

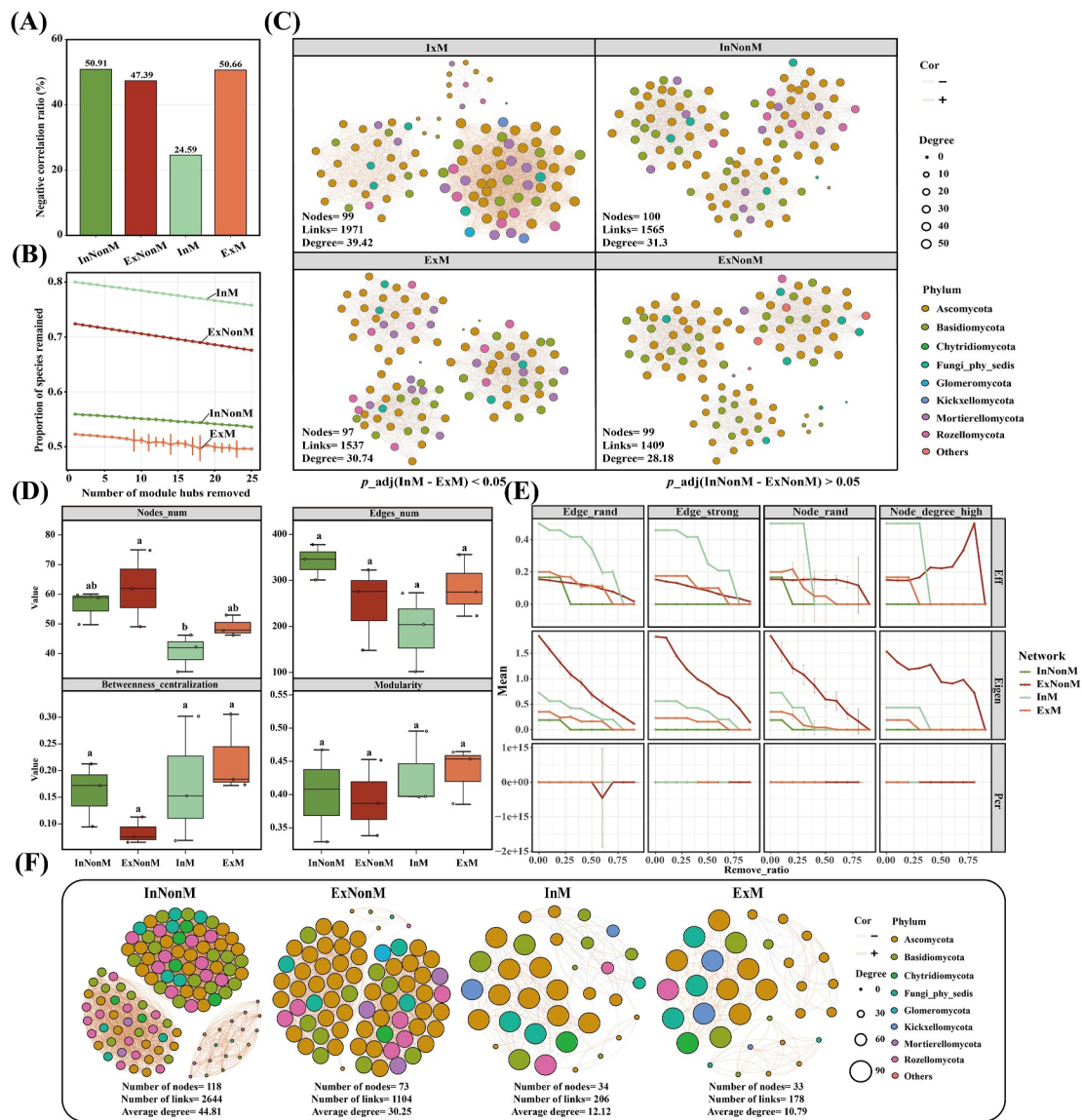


FIGURE 5 | Effects of ex situ conservation on fungal network topology in *P. purpuratum*. (A) Proportion of negative correlations among the top 100 OTUs. (B) Taxon retention rate as nodes are progressively removed. (C) Abundance-based subnetworks of the top 100 OTUs (nodes colored by phylum, sized by degree), showing node count, edge number, and average degree; $p < 0.05$ for in situ versus ex situ mycorrhizal groups; $p > 0.05$ for non-mycorrhizal comparisons. (D) Topological parameters including scale-free fit, betweenness centrality, and modularity; different lowercase letters indicate significant differences ($p < 0.05$). (E) Robustness tests under random and targeted removal of edges and nodes. (F) Subnetworks constructed from all OTUs (nodes colored by phylum, sized by degree).

be further optimized. The focus should shift from merely securing reproductive output to comprehensively optimizing the plant's physiological, symbiotic, and environmental adaptability.

4.2 | Rewiring of Host-Fungal Interaction Ecology by Conservation Practices

Ex situ conservation fundamentally has reshaped the underground ecological network of *P. purpuratum*, altering both fungal community composition [38, 39] and key host functional traits [40, 41]. When comparing the two habitats using redundancy analysis (RDA) and random forest models (Figure 6A–B), an interesting pattern emerges. In the native habitat, plants exhibit a classic “stress-response” physiological profile: high

osmotic adjustment (SS), strong antioxidant capacity (SOD), and a distinct $\delta^{15}\text{N}$ signature [42–44]. Interestingly, this physiological state correlates positively with the presence of certain beneficial fungi, (e.g., *Ramicandelaber* sp. [45]), as the plants appear to associate with these microbial partners to cope with environmental challenges. In the ex situ habitat, the host phenotypic profile was reconfigured. Enhanced water-use efficiency ($\delta^{13}\text{C}$) was accompanied by increased oxidative stress (MDA) [46, 47], a pattern likely stemming from the stable microclimate that imperfectly replicates the native light-temperature regime. Concurrently, this altered environment has selected for both mutualistic and pathogenic interactions: the host's association with stress-mitigating fungi (e.g., *Penicillium* sp. [48]) has strengthened, whereas the influence of potential pathogens (e.g., *Fusarium oxysporum* [49]) has been

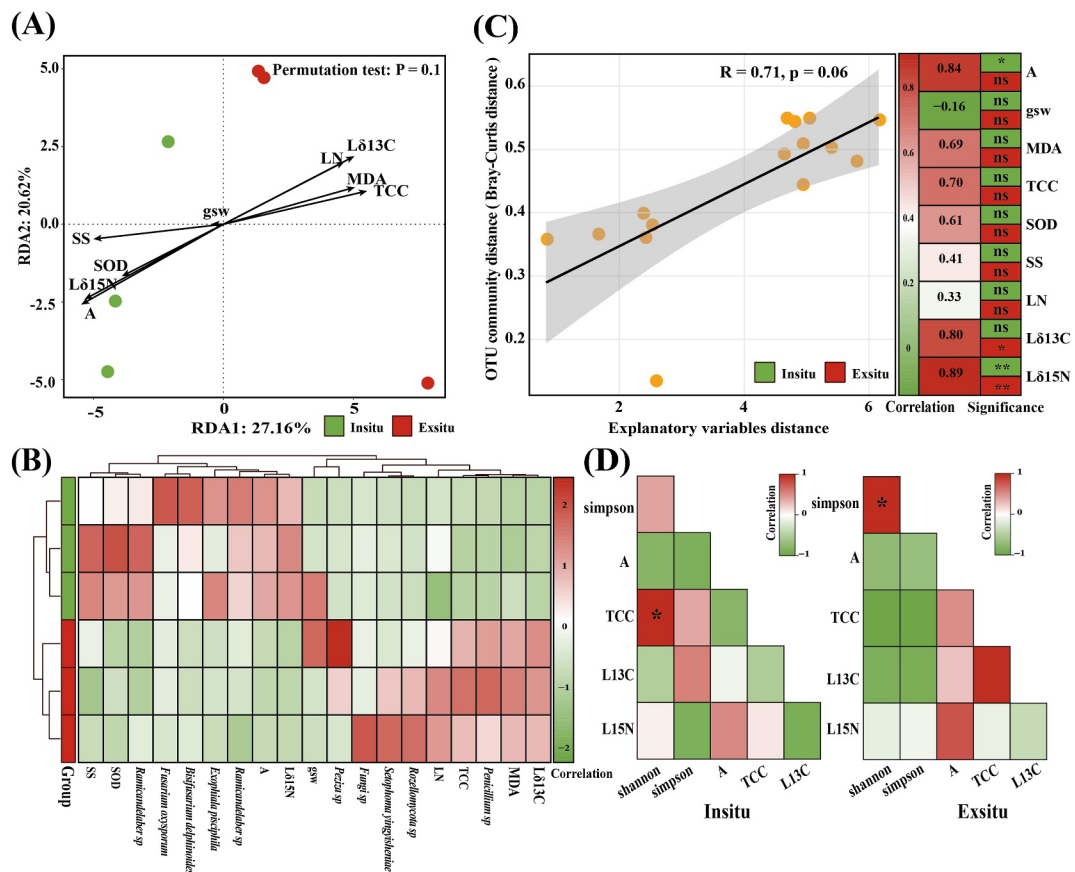


FIGURE 6 | Association characteristics between host phenotype and fungal communities under in situ/ex situ conservation. (A) RDA of host indicators and conservation groups. Points represent in situ (green) and ex situ (red) samples; arrows show VIF-screened host indicators. The permutation test p value is 0.1; (B) Correlation heatmap of the top 10 fungal taxa, host indicators, and conservation groups. Rows/columns include random-forest-selected taxa, host indicators, and group. Color denotes correlation coefficients (green: negative; red: positive). (C) Mantel test between host phenotypic and fungal community distances. Scatter plot shows Bray–Curtis versus host-phenotypic distances ($R = 0.71$, $p = 0.06$). Right heatmap displays Mantel r values for single indicators against Group (darker color: stronger association; e.g., leaf $\delta^{15}\text{N}$, $p < 0.01$). (D) Within-group correlations between host indicators and fungal diversity. Green/red blocks correspond to in situ/ex situ groups. Color intensity shows Spearman's correlation coefficient; asterisks (*) indicate $p < 0.05$. A reversal in correlation direction (e.g., for total chlorophyll content) is evident between groups.

modulated [50, 51]. These parallel shifts underscore how environmental conditions systemically reshape host-microbe interactions. Mantel tests confirmed host phenotype as the key driver of fungal community assembly ($R = 0.71$, $p = 0.06$) [52, 53]. Among host traits, leaf $\delta^{15}\text{N}$ stands out as the strongest predictor ($p < 0.01$) [54], suggesting that the plant's nitrogen metabolism may be actively selecting its microbial partners [55–57]. The correlation between total chlorophyll content and fungal diversity (Shannon index) reversed from positive in situ to negative ex situ (Figure 6D). Moreover, synchronized shifts in the Shannon and Simpson indices indicated a more even species distribution and a decline in previously dominant taxa, including key mycorrhizal fungi [58]. Collectively, these patterns differ from those typical of stable, healthy ecosystems [59]. These findings support an intriguing hypothesis: when a plant prioritizes allocating limited resources to photosynthesis, its capacity to maintain a high-diversity, mutually beneficial fungal network through “carbon investment” becomes constrained [60]. In other words, plants under ex situ conservation may exhibit a resource-allocation trade-off: securing their own photosynthetic function while simplifying their underground

symbiotic network. In summary, ex situ conservation represents more than a mere change of location; rather, it triggers a systemic reshaping of host-fungal interaction dynamics. Understanding this resource-allocation-driven mechanism provides an important logical foundation for our subsequent investigations into how mycorrhizal fungi coordinate functions within simplified networks (Section 4.3) and how non-mycorrhizal fungi provide essential auxiliary functions (Section 4.4).

4.3 | Multi-System Coordination Driven by Mycorrhizal Symbiosis

Amid the systemic reshaping triggered by ex situ conservation (see Section 4.2), the mycorrhizal fungal community exhibited a distinct response pattern relative to its non-mycorrhizal counterpart, laying the foundation for its role as a core stabilizer of the symbiotic system [61]. Key evidence demonstrated that the mycorrhizal community maintained high compositional, diversity, and structural stability following translocation: its dominant OTUs and alpha diversity showed no significant

changes, and it exhibited a smaller inter-habitat separation distance in PCoA analysis (Figure 3A–E) [62, 63]. Analysis of community assembly mechanisms further revealed that assembly was consistently dominated by stochastic processes, with an increased contribution from homogenizing dispersal—consistent with its high structural stability (Figure 4) [64, 65]. This stability did not imply rigidity but is accompanied by pronounced topological restructuring of the mycorrhizal network. Network analysis showed that the mycorrhizal network transitioned from a “single-core” to a “multi-small-cluster” structure, while negative correlations (putatively reflecting competitive interactions) within the network increased significantly (Figure 5A,C) [66]. This shift likely represents a strategy to enhance system resilience: by distributing functions across multiple relatively independent yet internally competitive sub-modules, reliance on any single keystone taxon is reduced, thereby preserving functional redundancy and stability of core functions amid environmental fluctuations [67]. Despite resource-allocation trade-offs (as discussed in Section 4.2), this stable yet restructured nature of the mycorrhizal network may act as a reliable “cornerstone” for the host to maintain basic nutrient acquisition under ex situ conditions [68], collectively supporting the adaptive hypothesis of “prioritizing core symbiotic functions under global resource constraints” [69–71].

4.4 | Auxiliary Adaptive Mechanisms of Non-Mycorrhizal Fungi and Photosynthetic Physiology

In sharp contrast to the stable mycorrhizal core described above, the non-mycorrhizal fungal community exhibited high plasticity in response to environmental changes, functioning as a dynamic auxiliary layer in the host’s adaptation process [72]. Following translocation, this community exhibited significantly increased species richness, underwent substantial compositional turnover, and its dominant taxa shifted from potential pathogens to groups with biocontrol potential (Figure 3A–D) [73]. Its assembly mechanism was more strongly influenced by deterministic environmental filtering and dispersal limitation (Figure 4B–C) [74], indicating that its assembly is more strongly driven by specific abiotic and biotic factors of the ex situ environment [75]. This intense community turnover may fulfill rapid environmental sensing and buffering functions; for instance, it alleviates pathogen pressure through mechanisms such as nutrient competition [76], thereby providing the host with a rapidly responsive external buffer zone [77].

In summary, the adaptation strategy of *P. purpuratum* under ex situ conservation exhibits a clear division of labor: a structurally reorganized yet dynamically stable mycorrhizal core is responsible for maintaining the foundational and reliable framework of symbiotic mutualism [71]; a high-turnover, strongly environment-responsive non-mycorrhizal community, in contrast, constitutes the dynamic auxiliary layer for coping with external stress fluctuations [78]. This integrated “stable core framework and dynamic peripheral response” model may be the key to its successful adaptation to the novel ex situ environment [79].

4.5 | Integrated Optimization Pathways and Research Prospects

Building on these findings, the ex situ conservation of *P. purpuratum* should evolve from simple environmental manipulation to integrated multisystem optimization. Progress can be made on three fronts:

First, maintain core symbiotic function. This requires continuous artificial inoculation with compatible mycorrhizal fungi and optimization of the soil ammonium-to-nitrate ratio to reduce resource competition [80, 81]. Implementing dynamic monitoring of fungal communities is also critical to regularly assess the stability of the symbiotic system.

Second, implement precise microenvironmental regulation. The focus should be on simulating natural light and humidity cycles and constraining the range of temperature fluctuations [82, 83]. Notably, the soluble sugar accumulation pattern observed in this study resembles a cold-stress response [82, 83], suggesting that current temperature settings in protective facilities may need further refinement.

Third, address reproductive dependencies. Efforts are needed to identify effective pollinators and to pre-inoculate compatible fungi during sterile sowing to improve seedling mycorrhization colonization success [32, 33, 37, 84]. This study has several limitations, two of which are particularly critical. First, the specific identities of effective pollinators and key functional fungi remain unresolved, hindering the construction of a complete interaction network. Second, the absence of long-term dynamic data makes it difficult to determine whether the observed phenotypic and symbiotic shifts represent a transient transitional state or a stable adaptive equilibrium.

Therefore, future conservation practices and research must be tightly integrated. While implementing the optimization strategies outlined above, priority should be given to (1) applying metagenomic sequencing to identify key mycorrhizal taxa and decipher pollination-symbiosis coupling relationships; (2) implementing longitudinal monitoring for at least 5 years; and (3) developing a precision conservation framework that integrates pollination management and symbiosis regulation. Only through such sustained feedback between research and practice can we more effectively promote the survival and sustainable growth of *P. purpuratum* in ex situ conditions.

5 | Conclusions

This integrated study demonstrates that ex situ conservation of *P. purpuratum* triggers a system-level adaptation shaped by resource allocation trade-offs. While achieving high reproductive success, the host prioritizes photosynthetic function, which in turn leads to a simplified mycorrhizal network with a restructured multi-cluster topology—one that ensures stable nutrient acquisition. Concurrently, non-mycorrhizal fungi form a dynamic, environment-responsive peripheral layer enriched with beneficial taxa. The mycorrhizal symbiosis centrally

coordinates this adaptative process across the host's reproductive and metabolic systems. These findings advocate for a shift in conservation strategies: moving from merely ensuring host survival to actively managing the reconstructed host-microbiome system through integrated symbiont inoculation, physiology-informed microenvironment regulation, and resolution of reproductive dependencies resolution.

Author Contributions

Yong Tan: writing – review and editing, writing – original draft, visualization, methodology, investigation, formal analysis, data curation, conceptualization. **Junxi Liang:** validation, resources. **Wentao Wang:** resources, project administration. **Jianing Tian:** data curation, investigation. **Qifei Yi:** writing – review and editing, writing – original draft, visualization, validation, supervision, resources, project administration, methodology, funding acquisition, data curation, conceptualization.

Acknowledgments

The authors would like to thank the Baixi Provincial Nature Reserve, Guangdong Province, China, for providing plant materials and experimental facilities for this study.

Funding

This work was supported by the South China Botanical Garden, Chinese Academy of Sciences, independent basic research project (*Paphiopedilum purpuratum*) (JCYJXM-2025012), Guangdong Science and Technology Plan Project (2022B111230001), and Guangdong Province Forestry Science and Technology Innovation Project (2024KJCX013).

Ethics Statement

Field sample collection were approved and conducted in compliance with the relevant national laws and local regulations.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

1. M. Kasso and M. Balakrishnan, "Ex Situ Conservation of Biodiversity With Particular Emphasis to Ethiopia," *ISRN Biodiversity* 2013 (2013): 1–11, <https://doi.org/10.1155/2013/985037>.
2. Y. Xu and R. Zang, "Conservation of Rare and Endangered Plant Species in China," *iScience* 26, no. 2 (2023): 106008, <https://doi.org/10.16/j.isci.2023.106008>.
3. S. Giaimo and A. Traulsen, "Generation Time Measures the Trade-Off Between Survival and Reproduction in a Life Cycle," *American Naturalist* 194, no. 2 (2019): 285–290, <https://doi.org/10.1086/704155>.
4. H. Liu, Z. Liu, X. Jin, et al., "Assessing Conservation Efforts Against Threats to Wild Orchids in China," *Biological Conservation* 243 (2020): 108484, <https://doi.org/10.1016/j.biocon.2020.108484>.

5. F. Yang, J. Gao, J. Li, et al., "The China Orchid Industry: Past and Future Perspectives," *Ornamental Plant Research* 4, no. 1 (2024), <https://doi.org/10.48130/opr-0023-0024>.
6. C.-M. Yeh, K. Chung, C.-K. Liang, and W.-C. Tsai, "New Insights Into the Symbiotic Relationship Between Orchids and Fungi," *Applied Sciences* 9, no. 3 (2019): 585, <https://doi.org/10.3390/app9030585>.
7. G. D. Tang, J. H. Ou, Y. B. Luo, X. Y. Zhuang, and Z. J. Liu, "A Review of Orchid Pollination Studies in China," *Journal of Systematics and Evolution* 52, no. 4 (2014): 411–422, <https://doi.org/10.1111/jse.12062>.
8. Z. Kovács, A. M. Csörgő, P. Csontos, and M. Hőhn, "Ex Situ Conservation in Botanical Gardens – Challenges and Scientific Potential Preserving Plant Biodiversity," *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 49, no. 2 (2021): 12334, <https://doi.org/10.15835/nbha49212334>.
9. D. Qian, M. Wang, Y. Niu, and Y. Xiang, "Sexual Reproduction in Plants Under High Temperature and Drought Stress," *Cell Reports* 44, no. 3 (2025): 115390, <https://doi.org/10.1016/j.celrep.2025.115390>.
10. M. F. Fay, "Orchid Conservation: How Can We Meet the Challenges in the Twenty-First Century?," *Botanische Studien* 59, no. 1 (2018): 16, <https://doi.org/10.1186/s40529-018-0232-z>.
11. R. P. Shefferson, T. Kull, M. J. Hutchings, et al., "Drivers of Vegetative Dormancy Across Herbaceous Perennial Plant Species," *Ecology Letters* 21, no. 5 (2018): 724–733, <https://doi.org/10.1111/ele.12940>.
12. L. Tikendra, A. M. Potshangbam, T. Amom, A. Dey, and P. Nongdam, "Understanding the Genetic Diversity and Population Structure of *Dendrobium Chrysotoxum* Lindl.-An Endangered Medicinal Orchid and Implication for Its Conservation," *South African Journal of Botany* 138 (2021): 364–376, <https://doi.org/10.1016/j.sajb.2021.01.002>.
13. D. J. Schoen, M. T. J. Johnson, and S. I. Wright, "The Ecology, Evolution, and Genetics of Plant Reproductive Systems," *New Phytologist* 224, no. 3 (2019): 999–1004, <https://doi.org/10.1111/nph.16222>.
14. X. Wan, D. Sun, and C. Gao, "Flower Opening Dynamics, Pollen-Ovule Ratio, Stigma Receptivity and Stigmatic Pollen Germination (in-vivo) in *Chaenomeles Speciosa* (Sweet) Nakai," *Scientific Reports* 14, no. 1 (2024): 7127, <https://doi.org/10.1038/s41598-024-57655-1>.
15. Y. Zhou and H. B. Shao, "The Responding Relationship Between Plants and Environment Is the Essential Principle for Agricultural Sustainable Development on the Globe," *Comptes Rendus Biologies* 331, no. 4 (2008): 321–328, <https://doi.org/10.1016/j.crv.2008.01.008>.
16. G. Losapio, "Contextualizing the Ecology of Plant-Plant Interactions and Constructive Networks," *AoB Plants* 15, no. 4 (2023): plad035, <https://doi.org/10.1093/aobpla/plad035>.
17. J. Hou, M. Liu, K. Yang, B. Liu, H. Liu, and J. Liu, "Genetic Variation for Adaptive Evolution in Response to Changed Environments in Plants," *Journal of Integrative Plant Biology* 67, no. 9 (2025): 2265–2293, <https://doi.org/10.1111/jipb.13961>.
18. Q. Liu, X.-L. Wang, P. M. Finnegan, and J.-Y. Gao, "Reproductive Ecology of *Paphiopedilum Spicerianum*: Implications for Conservation of a Critically Endangered Orchid in China," *Global Ecology and Conservation* 23 (2020): e01063, <https://doi.org/10.1016/j.gecco.2020.e01063>.
19. G. Qin, Q. Yuan, D. Xie, and C. Shi, "Effective Pollinators by Chinese Honeybee and Bumblebee in *Paphiopedilum Malipoense*," *International Journal of Agriculture and Biology* 22, no. 6 (2025), <https://doi.org/10.17957/ijab/15.1241>.
20. F. Tian, X. F. Liao, L. H. Wang, et al., "Isolation and Identification of Beneficial Orchid Mycorrhizal Fungi in *Paphiopedilum barbigerrum* (Orchidaceae)," *Plant Signaling & Behavior* 17, no. 1 (2022): 2005882, <https://doi.org/10.1080/15592324.2021.2005882>.
21. D. Wang, V. S. F. T. Merckx, H. Jacquemyn, and S. I. F. Gomes, "Mycorrhizal Communities in Orchidaceae Are Likely Shaped by Plant

- Trophic Mode and Biogeography But Not Phylogeny,” *Plant Diversity* 48, no. 1 (2025): 117–127, <https://doi.org/10.1016/j.pld.2025.08.002>.
22. T. Tsogtsaikhan, X. Yang, R. Gao, et al., “Biomass Allocation Between Reproductive and Vegetative Organs of *Artemisia* Along a Large Environmental Gradient,” *BMC Plant Biology* 25, no. 1 (2025): 27, <https://doi.org/10.1186/s12870-024-06030-3>.
 23. A. Carta, E. Fernández-Pascual, and G. Ottaviani, “We Need a Multidimensional View of the Plant Reproduction Continuum,” *Functional Ecology* 39, no. 3 (2024): 656–663, <https://doi.org/10.1111/1365-2435.14735>.
 24. J. Xia, W. Yuan, Y. P. Wang, and Q. Zhang, “Adaptive Carbon Allocation by Plants Enhances the Terrestrial Carbon Sink,” *Scientific Reports* 7, no. 1 (2017): 3341, <https://doi.org/10.1038/s41598-017-03574-3>.
 25. A. Wingler and S. Soualieu, “Overcoming Physiological Trade-Offs Between Flowering Time and Crop Yield: Strategies for a Changing Climate,” *Journal of Experimental Botany* 76, no. 10 (2025): 2646–2658, <https://doi.org/10.1093/jxb/eraf110>.
 26. G. C. Pagnussat and D. F. Gomez-Casati, “Plant Development and Reproduction in a Changing Environment,” *Journal of Experimental Botany* 75, no. 14 (2024): 4167–4170, <https://doi.org/10.1093/jxb/erae285>.
 27. G. Gebauer and M. Meyer, “(15) N and (13) C Natural Abundance of Autotrophic and Myco-Heterotrophic Orchids Provides Insight Into Nitrogen and Carbon Gain From Fungal Association,” *New Phytologist* 160, no. 1 (2003): 209–223, <https://doi.org/10.1046/j.1469-8137.2003.00872.x>.
 28. K. Suetsugu, J. Matsubayashi, and H. Okada, “Stable Isotope Signatures Illuminate Diverse Nutritional Strategies in Rhizoctonias and Their Orchid Partners,” *Plant, Cell and Environment* 48, no. 1 (2025): 792–804, <https://doi.org/10.1111/pce.15167>.
 29. B. Gravendeel and A. Dirks-Mulder, “Floral Development: Lip Formation in Orchids Unravelling,” *Nature Plants* 1, no. 5 (2015): 15056, <https://doi.org/10.1038/nplants.2015.56>.
 30. A. Jmrby, G. Martin, and S. Marlies, “A Cognitive Analysis of Deceptive Pollination: Associative Mechanisms Underlying Pollinators’ Choices in Non-Rewarding Colour Polymorphic Scenarios,” *Scientific Reports* 10, no. 1 (2020): 9476, <https://doi.org/10.1038/s41598-020-66356-4>.
 31. J. D. Rejon, F. Delalande, C. Schaeffer-Reiss, et al., “The Plant Stigma Exudate: A Biochemically Active Extracellular Environment for Pollen Germination?,” *Plant Signaling & Behavior* 9, no. 4 (2014): e28274, <https://doi.org/10.4161/psb.28274>.
 32. Y. Chen, C. Zhang, X.-f. Wang, and C.-Q. Ao, “Fertilisation of Polar Nuclei and Formation of Early Endosperms in *Dendrobium Catenatum*: Evidence for the Second Fertilisation in Orchidaceae,” *Australian Journal of Botany* 66, no. 4 (2018): 354–359, <https://doi.org/10.1071/bt17211>.
 33. A. S. Ryabchenko, G. L. Kolomeitseva, A. V. Babosha, and V. A. Koval, “Megasporogenesis, Megagametogenesis and Embryogenesis of *Liparis elliptica* (Orchidaceae). With Special Note to the Development of Unique Unitegmal Ovule,” *Protoplasma* 261, no. 3 (2024): 411–424, <https://doi.org/10.1007/s00709-023-01906-x>.
 34. N. N. Cabral, R. Pescador, M. V. M. Pinheiro, et al., “Different Spectral Qualities Do Not Influence the in Vitro and Ex Vitro Survival of *Epidendrum denticulatum* Barb. Rod.: A Brazilian Orchid,” *Vegetos* 36, no. 2 (2022): 427–441, <https://doi.org/10.1007/s42535-022-00440-0>.
 35. T. M. Lima, S. F. Silva, R. V. Ribeiro, J. Sánchez-Vilas, and F. Pinheiro, “Salt Tolerance in a Neotropical Orchid in the Absence of Local Adaptation to Salt Spray,” *American Journal of Botany* 111, no. 7 (2024): e16373, <https://doi.org/10.1002/ajb2.16373>.
 36. A. T. Patil, S. G. Mahadik, A. V. Mane, et al., “Effect of Plant Growth Regulators on Morpho-Physiological Parameters of Okra (*Abelmoschus esculentus* (L.) Moench) in Konkarn Condition,” *International Journal of Research in Agronomy* 7, no. 12 (2024): 26–30, <https://doi.org/10.33545/2618060x.2024.v7.i12a.2080>.
 37. J. Tao, F. Dong, Y. Wang, T. Xu, H. Chen, and M. Tang, “Arbuscular Mycorrhizal Fungi Alter Carbon Metabolism and Invertase Genes Expressions of *Populus simonii* X *P. nigra* Under Drought Stress,” *Physiologia Plantarum* 176, no. 5 (2024): e14572, <https://doi.org/10.1111/ppl.14572>.
 38. A. K. Alzarhani, D. R. Clark, G. J. C. Underwood, H. Ford, T. E. A. Cotton, and A. J. Dumbrell, “Are Drivers of Root-Associated Fungal Community Structure Context Specific?,” *ISME Journal* 13, no. 5 (2019): 1330–1344, <https://doi.org/10.1038/s41396-019-0350-y>.
 39. J. A. Hogan, M. A. Jusino, M. E. Smith, et al., “Root-Associated Fungal Communities Are Influenced More by Soils than by Plant-Host Root Traits in a Chinese Tropical Forest,” *New Phytologist* 238, no. 5 (2023): 1849–1864, <https://doi.org/10.1111/nph.18821>.
 40. J. W. Schroeder, J. T. Martin, D. F. Angulo, et al., “Host Plant Phylogeny and Abundance Predict Root-Associated Fungal Community Composition and Diversity of Mutualists and Pathogens,” *Journal of Ecology* 107, no. 4 (2019): 1557–1566, <https://doi.org/10.1111/1365-2745.13166>.
 41. X.-C. Li, X. Qian, C. Gao, et al., “Plant Identity Strongly Structures the Root-Associated Fungal Community in a Diverse Subtropical Forest,” *Basic and Applied Ecology* 55 (2021): 98–109, <https://doi.org/10.1016/j.baee.2021.01.002>.
 42. M. J. Rao, M. Duan, C. Zhou, et al., “Antioxidant Defense System in Plants: Reactive Oxygen Species Production, Signaling, and Scavenging During Abiotic Stress-Induced Oxidative Damage,” *Horticulturae* 11, no. 5 (2025): 477, <https://doi.org/10.3390/horticulturae11050477>.
 43. I. Couee, C. Sulmon, G. Gouesbet, and A. El Amrani, “Involvement of Soluble Sugars in Reactive Oxygen Species Balance and Responses to Oxidative Stress in Plants,” *Journal of Experimental Botany* 57, no. 3 (2006): 449–459, <https://doi.org/10.1093/jxb/erj027>.
 44. Y. Zhou, X. Cheng, J. Fan, and W. Harris, “Patterns and Controls of Foliar Nitrogen Isotope Composition on the Qinghai-Tibet Plateau, China,” *Plant and Soil* 406, no. 1–2 (2016): 265–276, <https://doi.org/10.1007/s11104-016-2882-5>.
 45. T. Zhou, J. Xu, J. Tan, et al., “Woody Peat Application Enriches Beneficial Soil Microorganisms to Enhance Aromatic Compound Accumulation in Tobacco Leaves,” *Applied Soil Ecology* 218 (2026): 106714, <https://doi.org/10.1016/j.apsoil.2025.106714>.
 46. W. T. Ma, Y. Z. Yu, X. Wang, and X. Y. Gong, “Estimation of Intrinsic Water-Use Efficiency From Delta(13)C Signature of C(3) Leaves: Assumptions and Uncertainty,” *Frontiers of Plant Science* 13 (2022): 1037972, <https://doi.org/10.3389/fpls.2022.1037972>.
 47. S. He, G. Liu, and H. Yang, “Water Use Efficiency by Alfalfa: Mechanisms Involving Anti-Oxidation and Osmotic Adjustment Under Drought,” *Russian Journal of Plant Physiology* 59, no. 3 (2012): 348–355, <https://doi.org/10.1134/s1021443712020033>.
 48. Y. Tao, D. Yi, Y. Wu, G. Deng, Z. Wang, and M. Peng, “Integrated Multi-Omics Reveal the Multidimensional Mechanisms of *Penicillium* Sp. in Selenite Biodegradation,” *Ecotoxicology and Environmental Safety* 309 (2025): 119588, <https://doi.org/10.1016/j.ecoenv.2025.119588>.
 49. C. Pan, F. Wang, Y. Chu, et al., “Targeting the Snf1 Kinase by Cinnamaldehyde Induces Glucose Starvation and Oxidative Stress in *Fusarium solani* for the Control of Sweet Potato Postharvest Decay,” *Postharvest Biology and Technology* 234 (2026): 114116, <https://doi.org/10.1016/j.postharvbio.2025.114116>.
 50. M. Caballol, M. A. Redondo, N. Catalan, et al., “Climate Acts as an Environmental Filter to Plant Pathogens,” *ISME Journal* 18, no. 1 (2024): wrae010, <https://doi.org/10.1093/ismejo/wrae010>.

51. C. Wagg, K. Schlaeppi, S. Banerjee, E. E. Kuramae, and M. G. A. Van Der Heijden, "Fungal-Bacterial Diversity and Microbiome Complexity Predict Ecosystem Functioning," *Nature Communications* 10, no. 1 (2019): 4841, <https://doi.org/10.1038/s41467-019-12798-y>.
52. A. S. Noble, J. Abbaszadeh, and C. K. Lee, "Host Selection Is Not a Universal Driver of Phyllosphere Community Assembly Among Ecologically Similar Native New Zealand Plant Species," *Microbiome* 13, no. 1 (2025): 35, <https://doi.org/10.1186/s40168-024-02000-x>.
53. C. Xiong, B. K. Singh, J. Z. He, et al., "Plant Developmental Stage Drives the Differentiation in Ecological Role of the Maize Microbiome," *Microbiome* 9, no. 1 (2021): 171, <https://doi.org/10.1186/s40168-021-01118-6>.
54. W. Song and Y. Zhou, "Linking Leaf $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ With Soil Fungal Biodiversity, Ectomycorrhizal and Plant Pathogenic Abundance in Forest Ecosystems of China," *Catena* 200 (2021): 105176, <https://doi.org/10.1016/j.catena.2021.105176>.
55. T. Zhou, J. Sun, and P. Shi, "Plant-Microbe Interactions Regulate the Aboveground Community Nitrogen Accumulation Rate in Different Environmental Conditions on the Tibetan Plateau," *Catena* 204 (2021): 105407, <https://doi.org/10.1016/j.catena.2021.105407>.
56. K. Tao, S. Kelly, and S. Radutoiu, "Microbial Associations Enabling Nitrogen Acquisition in Plants," *Current Opinion in Microbiology* 49 (2019): 83–89, <https://doi.org/10.1016/j.mib.2019.10.005>.
57. H. Koch and A. Sessitsch, "The Microbial-Driven Nitrogen Cycle and Its Relevance for Plant Nutrition," *Journal of Experimental Botany* 75, no. 18 (2024): 5547–5556, <https://doi.org/10.1093/jxb/erae274>.
58. B. B. Liang, W. J. Wang, X. X. Fan, et al., "Arbuscular Mycorrhizal Fungi Can Ameliorate Salt Stress in *Elaeagnus angustifolia* by Improving Leaf Photosynthetic Function and Ultrastructure," *Plant Biology* 23, no. Suppl 1 (2021): 232–241, <https://doi.org/10.1111/plb.13164>.
59. Z. Xu, X. Guo, W. J. Allen, et al., "Soil Fungi Influence the Relationship Between Plant Diversity and Ecosystem Multifunctionality," *Nature Communications* 16, no. 1 (2025): 5521, <https://doi.org/10.1038/s41467-025-60661-0>.
60. M. M. Chaves, J. Flexas, and C. Pinheiro, "Photosynthesis Under Drought and Salt Stress: Regulation Mechanisms From Whole Plant to Cell," *Annals of Botany* 103, no. 4 (2009): 551–560, <https://doi.org/10.1093/aob/mcn125>.
61. F. M. Martin and M. G. A. van der Heijden, "The Mycorrhizal Symbiosis: Research Frontiers in Genomics, Ecology, and Agricultural Application," *New Phytologist* 242, no. 4 (2024): 1486–1506, <https://doi.org/10.1111/nph.19541>.
62. F. T. Oktalira, M. R. Whitehead, and C. C. Linde, "Mycorrhizal Specificity in Widespread and Narrow-Range Distributed *Caladenia* Orchid Species," *Fungal Ecology* 42 (2019): 100869, <https://doi.org/10.1016/j.funeco.2019.100869>.
63. M. K. McCormick and H. Jacquemyn, "What Constrains the Distribution of Orchid Populations?," *New Phytologist* 202, no. 2 (2013): 392–400, <https://doi.org/10.1111/nph.12639>.
64. W. Liu, Z. Hou, X. Zhang, et al., "Stochastic Processes Dominate Bacterial and Fungal Community Assembly in Ultra-High-Altitude Areas of Southeast Tibet," *Ecological Indicators* 173 (2025): 113394, <https://doi.org/10.1016/j.ecolind.2025.113394>.
65. X. Huo, C. Ren, D. Wang, et al., "Microbial Community Assembly and Its Influencing Factors of Secondary Forests in Qinling Mountains," *Soil Biology and Biochemistry* 184 (2023): 109075, <https://doi.org/10.1016/j.soilbio.2023.109075>.
66. S. Sheykhal, J. Fernandez-Gracia, A. Traveset, et al., "Robustness to Extinction and Plasticity Derived From Mutualistic Bipartite Ecological Networks," *Scientific Reports* 10, no. 1 (2020): 9783, <https://doi.org/10.1038/s41598-020-66131-5>.
67. U. Brose, M. R. Hirt, R. Ryser, et al., "Embedding Information Flows Within Ecological Networks," *Nature Ecology & Evolution* 9, no. 4 (2025): 547–558, <https://doi.org/10.1038/s41559-025-02670-2>.
68. F. Walder, H. Niemann, M. Natarajan, M. F. Lehmann, T. Bollner, and A. Wiemken, "Mycorrhizal Networks: Common Goods of Plants Shared Under Unequal Terms of Trade," *Plant Physiology* 159, no. 2 (2012): 789–797, <https://doi.org/10.1104/pp.112.195727>.
69. B. S. Steidinger, "Mycorrhizal Arbitrage, a Hypothesis: How Mycoheterotrophs Could Profit From Inefficiencies in the Biological Marketplace," *Functional Ecology* 39, no. 6 (2024): 1431–1440, <https://doi.org/10.1111/1365-2435.14609>.
70. R. Salwan, R. Kaur, R. Sharma, and V. Sharma, "Mycorrhizal Symbioses: The Biological Trade-Off in Nutrient Cycling," in *Rhizosphere Revelations: Microbial Strategies for Sustainable Agriculture* (2025), 131–155, <https://doi.org/10.1016/bs.abr.2025.01.002>.
71. J. Shi, X. Wang, and E. Wang, "Mycorrhizal Symbiosis in Plant Growth and Stress Adaptation: From Genes to Ecosystems," *Annual Review of Plant Biology* 74, no. 1 (2023): 569–607, <https://doi.org/10.1146/annurev-arplant-061722-090342>.
72. B. Bai, W. Liu, X. Qiu, J. Zhang, J. Zhang, and Y. Bai, "The Root Microbiome: Community Assembly and Its Contributions to Plant Fitness," *Journal of Integrative Plant Biology* 64, no. 2 (2022): 230–243, <https://doi.org/10.1111/jipb.13226>.
73. Z. Shafi and M. Shahid, "Root Exudates as Molecular Architects Shaping the Rhizobacterial Community: A Review," *Rhizosphere* 36 (2025): 101212, <https://doi.org/10.1016/j.rhisph.2025.101212>.
74. I. Hayashi, H. Fujita, and H. Toju, "Deterministic and Stochastic Processes Generating Alternative States of Microbiomes," *ISME Commun* 4, no. 1 (2024): ycae007, <https://doi.org/10.1093/ismeco/ycae007>.
75. B. Yao, X. Mou, Y. Li, et al., "Distinct Assembly Patterns of Soil Bacterial and Fungal Communities Along Altitudinal Gradients in the Loess Plateau's Highest Mountain," *Microbial Ecology* 88, no. 1 (2025): 29, <https://doi.org/10.1007/s00248-025-02528-x>.
76. J. Sun, S. Xiao, and C. Xue, "The Tug-of-War on Iron Between Plant and Pathogen," *Phytopathology Research* 5, no. 1 (2023): 61, <https://doi.org/10.1186/s42483-023-00215-8>.
77. F. Ullah, S. Ali, M. Siraj, M. S. Akhtar, and W. Zaman, "Plant Microbiomes Alleviate Abiotic Stress-Associated Damage in Crops and Enhance Climate-Resilient Agriculture," *Plants* 14, no. 12 (2025): 1890, <https://doi.org/10.3390/plants14121890>.
78. H. A. Pantigoso, D. Newberger, and J. M. Vivanco, "The Rhizosphere Microbiome: Plant-Microbial Interactions for Resource Acquisition," *Journal of Applied Microbiology* 133, no. 5 (2022): 2864–2876, <https://doi.org/10.1111/jam.15686>.
79. W. Cai, J. Snyder, A. Hastings, and R. M. D'Souza, "Mutualistic Networks Emerging From Adaptive Niche-Based Interactions," *Nature Communications* 11, no. 1 (2020): 5470, <https://doi.org/10.1038/s41467-020-19154-5>.
80. H. Bücking and A. Kafle, "Role of Arbuscular Mycorrhizal Fungi in the Nitrogen Uptake of Plants: Current Knowledge and Research Gaps," *Agronomy* 5, no. 4 (2015): 587–612, <https://doi.org/10.3390/agronomy5040587>.
81. C. Wang and Y. Kuzyakov, "Soil Organic Matter Priming: The pH Effects," *Global Change Biology* 30, no. 6 (2024): e17349, <https://doi.org/10.1111/gcb.17349>.
82. H. B. Lee, S. J. Jeong, N. H. Lim, S. K. An, and K. S. Kim, "Correlation Between Carbohydrate Contents in the Leaves and Inflorescence Initiation in *Phalaenopsis*," *Scientia Horticulturae* 265 (2020): 109270, <https://doi.org/10.1016/j.scienta.2020.109270>.
83. E. Zhang, Y. Wang, T. W. Crowther, et al., "Mycorrhiza Increases Plant Diversity and Soil Carbon Storage in Grasslands," *Proceedings of*

the National Academy of Sciences of the USA 122, no. 7 (2025): e2412556122, <https://doi.org/10.1073/pnas.2412556122>.

84. J. F. Qarachal and M. Alizadeh, "Integrating Mycorrhizal Fungal Networks Into Plant Pathology: Mechanisms of Disease Suppression and Stress Tolerance in Tree Communities," *Physiological and Molecular Plant Pathology* 139 (2025): 102834, <https://doi.org/10.1016/j.pmpp.2025.102834>.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Figure S1: Intergroup differences in isotopic and physiological indicators of *P. purpuratum* under ex situ versus in situ conservation conditions. (A-1, A-2) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of autotrophic plants, *P. purpuratum*, and heterotrophic plants. Leaf traits of *P. purpuratum*: (A-3) $\delta^{13}\text{C}$; (A-7) $\delta^{15}\text{N}$; (A-4) carbon content (C%); (A-8) nitrogen content (N%). Root traits of *P. purpuratum*: (A-5) $\delta^{13}\text{C}$; (A-9) $\delta^{15}\text{N}$; (A-6) carbon content (C%); (A-10) nitrogen content (N%). (B-1) Absorbed radiation (Rabs); (B-2) Transpiration rate (E); (B-3) Chamber relative humidity (RHcham); (B-4) Leaf vapor pressure deficit (VPDleaf); (B-5) Stomatal conductance (gs_w); (B-6) Leaf temperature (Tleaf Cnd); (B-7) Inter-cellular CO_2 concentration (Ci); (B-8) Net photosynthetic rate (A); (C-1) Malondialdehyde (MDA) content; (C-2) Thiobarbituric acid reactive substances (TBA); (C-3) Total chlorophyll content (TCC); (C-4) Chlorophyll a/b ratio (Chl a/b Ratio); (C-5) Superoxide dismutase (SOD); (C-6) Antioxidant capacity (AOC); (C-7): Catalase (CAT); (C-8) Peroxidase (POD); (C-9) Soluble protein (SP); (C-10) Soluble sugar (SS). Data are presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; ns: not significant.